mRNA regulation

in the *C. elegans* germ line

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Abbreviations

A site	**aminoacyl site**
ARE	**AU rich element**
ARE-BP	**AU rich element binding protein**
CPE	**cytoplasmic polyadenylation element**
CPEB	**cytoplasmic polyadenylation element binding protein**
CPSF	**cytoplasmic polyadenylation specificity factor**
CTD	**C-terminal domain (of RNA Polymerase II)**
DTC	**distal tip cell**
E site	**exit site**
eEF2K	**eEF2 kinase**
EJC	**exon-junction complex**
GBM	**GLD-1 binding motif**
hnRNP	**heterologous nuclear ribonucleoprotein**
IRE	**iron response element**
IRES	**internal ribosome entry site**
IRP	**iron regulatory protein**
MAPK	**mitogen-activated protein kinase**
MBP	**myelin basic protein**
MBT	**mid-blastula transition**
miRISC	**miRNA dependent RNA induced silencing complex**
MSP	**major sperm protein**
NMD	**nonsense-mediated decay**
OET	**oocyte-to-embryo transition**
P site	**peptidyl site**
PABP	**polyA binding protein**
PUF	**Pumilio and FBF**
QR	**quaking related**
RBP	**RNA binding protein**
RNP	**ribonucleoprotein**
RRE	**RNA binding protein recognition element**
RRM	**RNA recognition motif**

STAR	**signal transduction and activation of RNA**
S6K	**S6 kinase**
TOR	**target of rapamycin**
TTP	**tristetraprolin**
uORF	**upstream open reading frame**
UTR	**untranslated region**
ZBP1	**zipcode binding protein 1**
ZGA	**zygotic gene activation**
4E-BP	**eIF4E binding protein**
4E-T	**eIF4E transporter**
5' TOP	**5' terminal oligopyrimidine tract**

1. Summary

The *C. elegans* germ line relies heavily on post-transcriptional regulation of gene expression but the scale of mRNA regulation in the germ line is still unknown. Germ cells initially divide mitotically, they then enter meiosis and finally differentiate into oocytes. Transcription ceases during oogenesis and does not get reactivated until the early embryo. The oocyte-to-embryo transition (OET) encompassing oocyte maturation, fertilization and early embryogenesis, therefore solely depends on maternal factors. Maternal mRNA storage describes the repression and stabilization of these factors until they are needed. At the four-cell stage, somatic blastomeres become dependent on zygotic transcription and at the same time a subgroup of maternal mRNAs (class II maternal mRNAs) gets specifically degraded.

Many developmental decisions in the germ line are regulated by RNA binding proteins (RBPs). A crucial regulator is the STAR domain protein GLD-1, which is expressed in the central gonad. GLD-1 regulates many of the developmental decisions in the germ line and loss of GLD-1 prevents oogenesis and leads instead to the development of a proliferative tumor. GLD-1 binds a large number of mRNAs, and is known to repress the translation of various transcripts but the mechanism by which it does so is unknown.

We found that translation initiation of many germline mRNAs is repressed, and that GLD-1 globally represses translation initiation of its targets. Importantly, we revealed an additional role of GLD-1 in stabilizing a large number of its bound mRNAs, suggesting that GLD-1 plays a central role in maternal mRNA storage. While we couldn't detect an interaction between GLD-1 and translation initiation factors, we observed that GLD-1 associates with components of a conserved germline RNP complex. These components include the polyA binding protein (PABP), Y-box proteins, the Sm-like protein CAR-1 and the DDX6 helicase CGH-1, which has recently been implicated in maternal mRNA protection. Interestingly we found that while CGH-1 does not influence the translational repression of investigated GLD-1 targets, CGH-1 and GLD-1 stabilize a common set of transcripts. Remarkably, these co-regulated messages nearly exclusively encode for mRNAs that are required for the oocyte-to-embryo transition. We therefore propose a two-step model where GLD-1 binding prevents translation initiation and primes many targets for CGH-1-dependent mRNA stabilization, ultimately leading to mRNA storage.

2. Introduction

The germ line is the only cell lineage that transmits genetic information to future generations. Germ cells are therefore considered to have an underlying totipotency. In the course of germ cell development, germ cells initially proliferate mitotically, they then enter and progress through meiosis before differentiating into mature gametes. Many of the developmental decisions in the germ line rely heavily on the post-transcriptional regulation of gene expression and loss of mRNA regulation can lead to severe germline defects including tumorigenesis and somatic transdifferentiation (Ciosk et al. 2006).

Furthermore, the OET, encompassing oocyte maturation, fertilization and early embryogenesis, is largely dependent on maternal mRNAs. The OET occurs while transcription is globally repressed, which is why maternal mRNAs accumulate during oocyte development. This situation implies the importance of maternal mRNA regulation. On one hand, the expression of maternal mRNAs needs to be temporally and spatially tightly regulated, to prevent precocious translation of mRNAs. At the same time, maternal transcripts need to be stabilized so they can get reactivated when needed. Maternal mRNA storage describes both the translation repression and the stabilization of these mRNAs and highlights once more the importance of post-transcriptional regulation of gene expression in the germ line.

2.1. The mRNA life cycle

An RNA molecule is never present in the cell in its naked form but is bound, throughout its life cycle, by many RBPs that impact on the maturation, translation, localization and degradation of the RNA.

2.1.1. mRNA processing and export

Until a mature mRNA molecule is formed, the pre-mRNA undergoes several processing events. 5'-capping, splicing and 3'-end polyadenylation are tightly coupled and influence numerous later steps during the lifecycle of an mRNA (Hocine et al. 2010). A key player of coordinating these co-transcriptionally occurring events is the C-terminal domain of RNA Polymerase II (CTD of RNA Pol II), which is heavily modified and acts as a scaffold for different processing factors (Fig. 1).

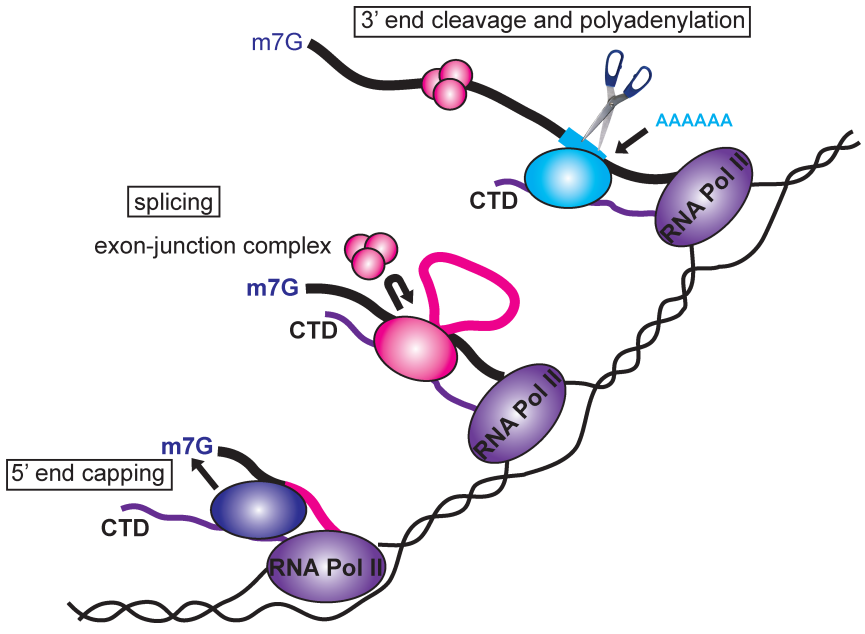

Figure 1
Transcription and RNA processing are tightly coupled processes. The CTD of RNA Pol II acts as a scaffold for different processing factors and thereby couples capping, splicing and 3' end processing to different steps of transcription.

2.1.1.1. Capping

Once 25 – 30 nucleotides of the pre-mRNA have been synthesized, the γ phosphate of the terminal nucleotide is hydrolyzed by an RNA triphosphatase. Next, a guanylyltransferase transfers GMP from GTP to the terminal nucleotide leading to the formation of GpppN, which then gets methylated at the N7 position by a methyltransferase. CTD modificiation during transcription initiation promote the association between the CTD and the guanylyltransferase, thereby coupling capping to translation initiation. The 7-methylguanosine cap and its bound proteins regulate several steps following capping. The cap-binding complex, which consists of the cap-binding proteins CBP-80/20 influences RNA splicing, 3'-end processing, and export (Izaurralde et al. 1995; Flaherty et al. 1997). In the cytoplasm, the 5'-cap structure protects the mRNA from 5' → 3' degradation and regulates mRNA recruitment to the ribosome by interacting with the translation initiation factor eIF4F. eIF4F additionally binds the PABP, mediating mRNA circularization and thereby enhancing translation initiation and protein synthesis (Hocine et al. 2010; Tarun and Sachs 1996).

2.1.1.2. Splicing

Succeeding capping, the nascent transcript is subjected to splicing, leading to the removal of introns, rejoining of exons and the deposition of the exon-junction complex (EJC). The spliceosome carries out two trans-esterification reactions, starting with the nucleolytic attack of the phosphodiester bond at the 5'-splice site by the 2'-hydroxyl group of the branch-point adenosine. The free hydroxyl group at the 5'-splice site then attacks the 3' splice nucleotide, thereby releasing the intron lariat. Also splicing is coupled to transcription and splicing factors are recruited to the RNA via the CTD of RNA Pol II. Introns are recognized via the branch-point and the 5' and 3' splice sites. But additional flanking sequences and many regulatory proteins determine which splice sites are ultimately selected. 92 – 94 % of all human transcripts undergo alternative splicing, which provides an additional layer of regulating gene expression and increasing proteome diversity. Also splicing impacts on later events in the mRNA life cycle. Spliced transcripts are bound by factors

mediating mRNA export and the EJC influences mRNA transport, translation initiation and – via the nonsense-mediated decay (NMD) pathway - controls mRNA quality (Hocine et al. 2010).

2.1.1.3. 3' end processing and export

The final step of processing begins with the endonucleolytic cleavage 10 – 30 nucleotides downstream of a signal sequence and is followed by polyadenylation at the 3'-site. Similar to alternative splicing, transcripts can undergo alternative polyadenylation, giving rise to different mRNA isoforms. And like the 5'-cap structure, the 3'-polyA tail is important for mRNA stability and translation.

Before an mRNA is exported into the cytoplasm it has to pass several mRNA quality control steps. Splicing defective mRNAs as well as transcripts with aberrant 3'-ends are retained at the site of transcription and directly degraded by the exosome in the nucleus. Once an mRNA has passed the nuclear surveillance system, mRNA export factors, which have been deposited on the mRNA during processing, interact with nuclear pore proteins and mediate the transport of the mature mRNA into the cytoplasm (Hocine et al. 2010).

2.1.2. mRNA translation

After an mRNA has been exported into the cytoplasm it is in principle accessible to the translation machinery. Translation can be divided into three steps – translation initiation, elongation and termination. The terms 40s, 43s, 48s, 60s and 80s correspond to Svedberg coefficients, describing the sedimentation rate of these particles during ultracentrifugation.

2.1.2.1. Translation initiation

In the canonical cap-dependent translation initiation pathway, the 5'cap structure of an mRNA is recognized by the eukaryotic initiation factors eIF4B and eIF4F (Fig. 2). eIF4F consists of three proteins: eIF4E, eIF4G and eIF4A. eIF4G acts as a scaffold for the helicase eIF4A, the cap-binding protein eIF4E, and additionally has binding sites for the PABP and eIF3. eIF4G thereby plays a central role in stabilizing the eIF4E-mRNA interaction, circularizing the mRNA and recruiting the small ribosomal subunit. The eIF4E-eIF4G interaction is crucial for translation initiation and is subjected to many regulatory events that will be discussed in detail below. The helicase eIF4A is stimulated by eIF4B and eIF4G and unwinds the 5'-cap proximal region of the mRNA, thereby preparing it for ribosomal attachment.

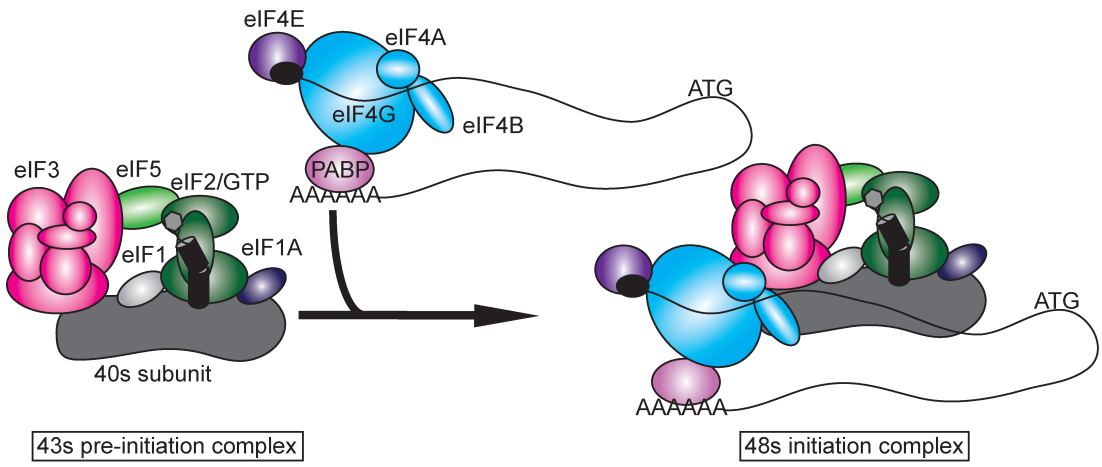

Figure 2
Formation of the 48s initiation complex. Translation initiation begins with the assembly of the ternary complex, eIF3, eIF5, eIF1/1A and the 40s ribosomal subunit into the 43s pre-initiation complex. eIF4G induces the closed-loop formation of the mRNA by interacting with the cap-binding protein eIF4E and the polyA binding protein PABP. Additionally eIF4G stimulates ribosome recruitment by binding eIF3. Once the 43s initiation complex is bound to an mRNA it starts scanning the 5' UTR for an ATG.

Ribosome recruitment begins with the assembly of the ternary complex composed of the initiator tRNA, Met-tRNA$_i$, and eIF2 bound to GTP. Next, the ternary complex with the small ribosomal subunit, eIF3, eIF5 and eIF1/1A, forms the 43s pre-initiation complex. The attachment of the 43s complex to the RNA leads to the formation of the 48s initiation complex. The joining of the two particles is mediated via an interaction between eIF3 and eIF4G and is enhanced by structures in the 5' untranslated region (5' UTR) of the mRNA.

As an alternative to the cap-dependent mechanism of translation initiation, the small ribosomal subunit can get recruited to mRNAs via internal ribosome entry sites (IRESs) located in the 5'-UTR. To date, the pathway is thought to be mostly important for the translation of viral RNAs and will therefore not be discussed any further.

Once the 43s complex is bound to the RNA, it scans the 5' UTR in a 5' to 3' direction until it reaches the first AUG start codon in an amenable context, whereas eIF1 is crucial for the fidelity of AUG recognition. Once the correct start codon has been recognized and the codon-anticodon base pairing has been established, eIF5 stimulates eIF2-GTP hydrolysis and P_i release, leading to the commitment of the arrested ribosome. The 60s ribosomal subunit joins together with GTP-bound eIF5B, causing the release of eIF2-GDP, eIF1, eIF3 and eIF5 (Fig. 3).

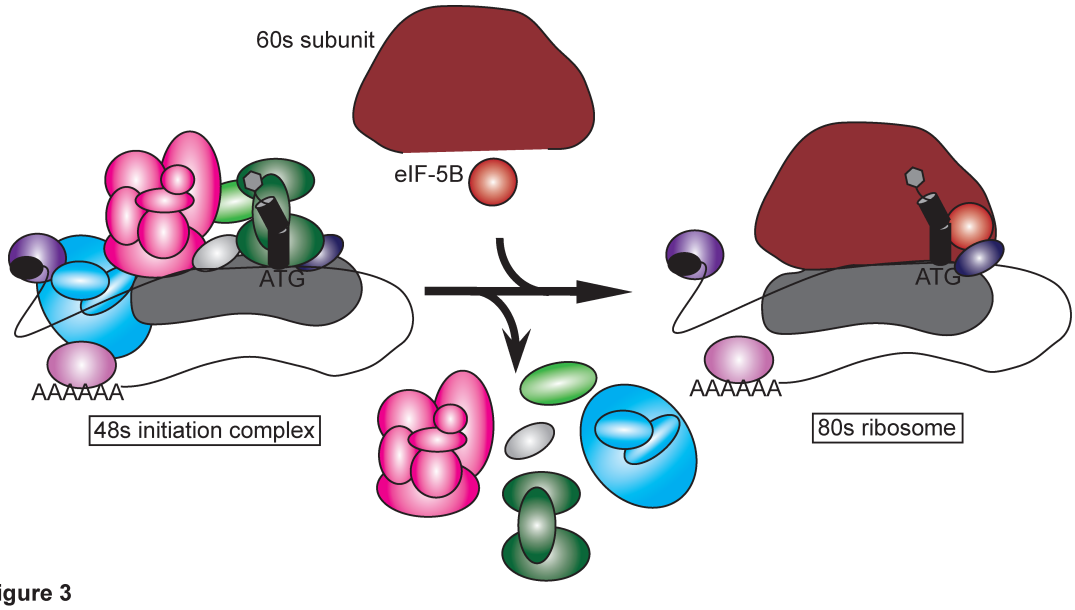

Figure 3
Assembly of the 80s ribosome. Once the 43s complex has recognized an ATG, the 60s ribosomal subunit joins, which caused the release of several initiation factors.

As soon as the 80s ribosome is assembled, eIF1A stimulates the GTPase activity of eIF5B, leading to the dissociation of both initiation factors. A frequent feature of eukaryotic mRNAs is the presence of upstream open reading frames (uORFs) in the 5'-UTR. Unless the uORF has a disproportional length or includes stable secondary RNA structures, the ribosome is often able to resume scanning. However, uORF recognition still causes the dissociation of the ternary eIF2 complex and the 60s subunit. The ternary complex is reacquired during scanning, rendering the 40s subunit

competent for reinitiation and the 60s subunit rejoins once the downstream AUG of the protein coding ORF has been recognized (Jackson et al. 2010).

2.1.2.2. Translation elongation and termination

Compared to translation initiation, translation elongation and termination are less complex and are less regulated than translation initiation. During translation elongation tRNAs get consecutively bound to the A (aminoacyl), P (peptidyl) and E (exit) sites of the ribosome. eEF1A brings aminoacyl-tRNAs under GTP consumption to the A site of the ribosome, where the tRNA anticodon pairs with the codon of the mRNA.

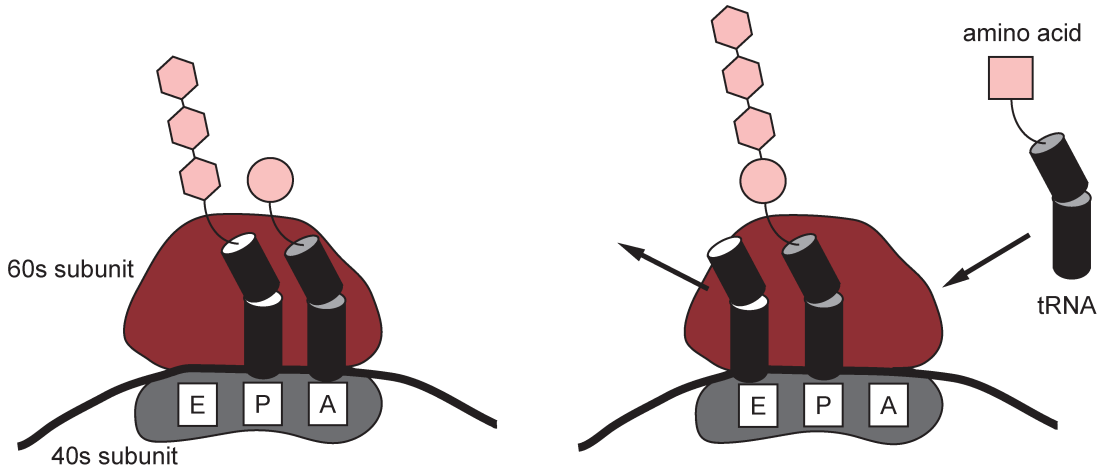

Figure 4
Translation elongation. Aminoacyl-tRNAs are brought to the A site of the ribosome. The peptidyl chain bound to the tRNA at the P site gets transfered to the amino acid of the aminoacyl-tRNA. The ribosome translocates to the next codon: the uncharged tRNA gets ejected from the E site, the peptidyl-tRNA is now placed at the P site and the A site is free to bind the next aminoacyl-tRNA.

The peptidyl-transferase activity of the ribosome promotes the formation of a peptide bond between the amino acid bound to the tRNA at the A site and the peptidyl chain bound to the tRNA at the P site. The ribosome is now ready to translocate to the next codon, which requires eEF2 and more GTP. Thereby the A site is free to bind another aminoacyl-tRNA, the P site is occupied by the tRNA, which was previously in the A position and the uncharged tRNA has been placed from the P to the E site, from where it gets ejected (Fig. 4). Once the ribosome encounters one of three stop codons,

the ribosome is bound by eukaryotic release factor 1 (eRF1) and eRF3-GTP. eRF1 acts as a tRNA mimic and binds the A position of the ribosome. GTP hydrolysis by eRF3 induces the hydrolysis of the ester bond at the P site peptidyl-RNA, which is followed by the release of the polypeptide chain. The dissociation of the release factors from the ribosome is promoted by eRF3, while ribosome recycling seems to be mediated by translation initiation factors. eIF3 ensures the splitting into ribosomal subunits and is enhanced by eIF1 and eIF1A. Additionally, eIF1 stimulates the release of tRNAs from the P site and eIF3γ causes mRNA dissociation (Alkalaeva et al. 2006; Pisarev et al. 2007).

To increase the rate of protein synthesis an mRNA molecule is bound and translated by several ribosomes at a time referred to as polyribosomes or polysomes. This process is facilitated by the circularization of eukaryotic mRNA molecules, since after translation termination, the dissociated ribosomal subunits are in an optimal position to reinitiate translation.

2.1.3. mRNA degradation

Once an mRNA has performed its task in the cell – it gets degraded by one of two major mRNA decay pathways.

As previously mentioned, during mRNA processing, mRNAs, with the exception of histone mRNAs, acquire a 5' cap structure and a 3' polyA tail. The cap-binding complex and the PABP bind to these structures respectively and induce the circularization of the mRNA, a process not only important to facilitate translation but also to protect mRNAs from nucleases. Both major eukaryotic mRNA decay pathways initiate mRNA degradation by shortening of the polyA tail. The Pan2-Pan3 complex mediates the trimming of nuclear polyA tails in yeast, but also stimulates the default deadenylation of transcripts upon mRNA export in mammalian cells. The Pan2-Pan3 complex degrades polyA tails in a distributive manner – i.e. the deadenylase hydrolyzes only few nucleotides before it associates with another RNA molecule. Whereas mRNA decay can still take place in the absence of Pan2-Pan3, the

Ccr-4-Caf1 complex is crucial for mRNA deadenylation. Ccr4-Caf1, once bound to an mRNA, completely hydrolyzes the polyA tail, acting as a processive deadenylase. Once the polyA tail has been shortened to a critical length, the mRNA is susceptible to get degraded by one of the two major eukaryotic mRNA decay pathways. On one hand, deadenylated transcripts can undergo decapping: the 3' end of an mRNA is first bound by the Lsm complex, which then allows the Dcp1-Dcp2 complex to hydrolyze the cap. Decapping leaves the mRNA with a 5' monophosphate and releases m^7GDP. Dcp1 stimulates the catalytically active subunit Dcp2, which contains, like certain pyrophosphatases, a Nudix motif. Decapped mRNAs can then be targeted by the 5' to 3' exonuclease Xrn1.

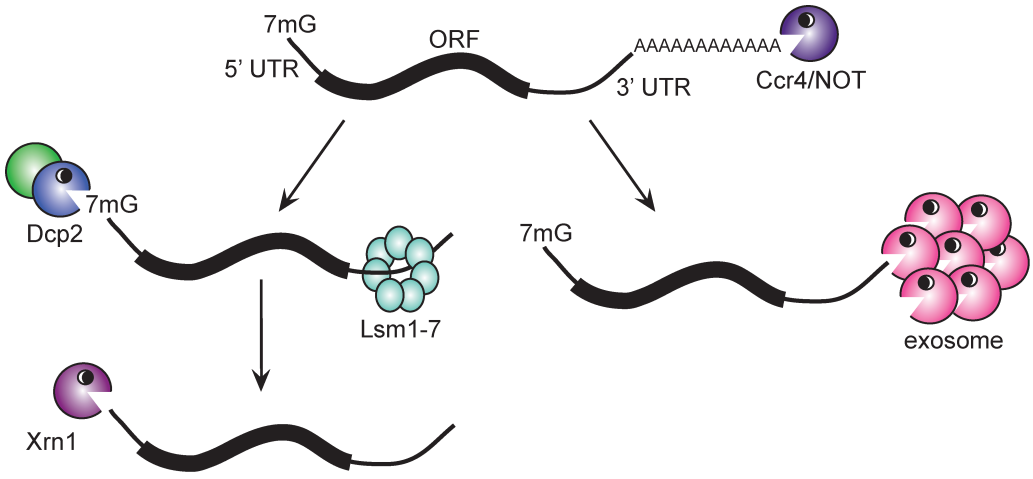

Figure 5
Eukaryotic mRNA decay. Both major mRNA decay pathways are initiated by Ccr4-Not mediated deadenylation. Deadenylated transcripts are either subject to decapping followed by 5' to 3' exonucleolytic digestion or alternatively, are degraded from the 3' end by the exosome.

Alternatively, deadenylated transcripts are exonucleolytically degraded from the 3' end by the exosome. Besides degrading cytoplasmic mRNAs, this multisubunit complex has several other functions related to RNA. Also 3'-end processing of precursor RNAs and nuclear mRNA degradation are mediated by the exosome. The exosome thus plays a major role in controlling mRNA processing, mRNA quality surveillance and mRNA degradation and thus plays a major function in regulating RNAs throughout their lifecycle (Parker and Song 2004; Houseley et al. 2006).

2.2. Post-transcriptional control of gene expression

While all cells of an organism contain identical genetic information, only a specific subset of proteins is expressed in each cell type to determine the function of the cell. Only if the correct amount and the correct set of proteins are synthesized at the right time, can cells function properly. For many years, it has been assumed that transcriptional regulation of genes is the major source of differential gene expression. However, it becomes more and more evident, that transcriptional regulation can only partly explain why and at what level proteins are expressed. Accordingly, quantitative mRNA expression studies are insufficient to predict protein levels (Gygi et al. 1999). Post-transcriptional control of gene expression comprises all mechanisms targeting the transcript once RNA polymerase has bound to the promoter of a gene. In contrast to transcriptional regulation, post-transcriptional regulation is thought to influence protein synthesis in a more direct and rapid way. The importance of this regulation of gene expression is manifested by the fact that deregulated mRNA translation is associated with many developmental defects and diseases including cancer (Silvera et al. 2010). Although all steps during the mRNA life cycle are tightly regulated and can influence protein synthesis, I will focus here on the regulatory mechanisms acting on the mature mRNA in the cytoplasm.

Repression of translation can be divided into two different categories. Firstly, translation can be globally repressed by impacting on translation factors or ribosomes. Alternatively, translational regulation is mediated by trans-acting factors, such as RBPs or small RNAs, which bind to specific cis elements in UTRs of an mRNA. This binding can then influence mRNA degradation, sequestration, localization and translation. Often, depending on the developmental stage and the cellular compartment, different trans acting factors employ distinct mechanisms on one mRNA, ensuring its temporal and spatial regulation.

2.2.1. Global mRNA repression

Protein synthesis is an energy consuming process and is therefore tightly regulated. A cell has to be able to adapt the rate of protein synthesis according to its needs and

under certain conditions needs to globally repress translation. As mentioned above most regulatory mechanisms affect translation by targeting the initiation step. With translation consuming a large amount of energy, it is sensible to control the first step of translation and since translation initiation is the most complex step of translation, it can get regulated in various ways.

For example, in response to various stress conditions, such as starvation, oxidative stress or double-stranded RNA, eIF2α gets phosphorylated. While under these circumstances, the ternary eIF2-GTP complex still forms, phosphorylated eIF2α sequesters the guanine exchange factor of eIF2, eIF2B. The decrease in eIF2B activity is followed by a reduction in ternary complex formation and therefore translation initiation (Bushman et al. 1993). Another example of a globally targeted initiation factor is eIF4E. Under inhibitory growth conditions, eIF4E binding proteins (4E-BPs) compete with eIF4G for eIF4E binding, thereby preventing translation initiation. Stimulation of cell growth leads to the activation of target of rapamycin (TOR), which subsequently phosphorylates 4E-BPs. The phosphorylation of 4E-BPs, prevents the association of 4E-BPs with eIF4E, allowing translation initiation to take place (Bushman et al. 1993). Several other events of globally controlling protein synthesis have been linked to cell growth. For example, TOR activity correlates with the translation activation of TOP mRNAs. TOP mRNAs contain 5' terminal oligopyrimidine tracts (5' TOP) in their 5' UTR and encode for many components of the translation machinery. Inhibition of cell growth leads to the coordinate repression of 5' TOP mRNA translation by various trans-acting factors (Levy et al. 1991). The mechanisms of how these trans-acting factors prevent translation initiation have yet to be determined.

A well-known case where translation elongation is inhibited is the regulation of eEF2. Under inhibitory growth conditions, eEF2 kinase (eEF2K) phosphorylates and thereby inactivates eEF2. In response to growth signals, TOR signaling activates S6 kinase (S6K). S6K in turn phosphorylates and inactivates the eEF2 inhibitor, eEF2K. Active eEF2 is then capable to promote translation elongation (Wang et al. 2001).
Other targets of S6K include eEF2, eIF4B and rpS6. Whereas S6K positively regulates protein synthesis by activating eEF2 and eIF4B, the phosphorylation of rpS6

negatively affects translation, suggesting that S6K plays a central role in the fine tuning of protein synthesis in response to growth stimulation (Ruvinsky and Meyuhas 2006).

2.2.2. Sequence-specific mRNA repression

Most regulatory sequences bound by trans-acting factors, are located within the 3' untranslated region of an mRNA.

2.2.2.1. Cis-acting elements

Although the 3' UTR in a linear RNA molecule is quite distant from the cap, the closed loop structure brings both features into close proximity of one another and thereby allows the 3' UTR to impact on translation initiation.

There are however few reports of regulatory sequences present in the 5' UTR. 5' TOP mRNAs have already been discussed in the context of global mRNA repression. Another example is the iron response element (IRE), which comprises stem-loops within the 5' UTR of ferritin mRNAs. Ferritin mRNAs encode for subunits of the iron storage protein and need to be translationally repressed in the absence of iron. Under these conditions, iron regulatory proteins (IRPs) bind to IREs. Since IRE's are located close to the cap structure, IRP binding sterically interferes with translation initiation by blocking the recruitment of the 43s ribosomal complex (Gray and Hentze 1994).

To date numerous cis elements located within 3' UTRs have been described. For instance AU rich element (ARE) are found in mRNAs encoding for cytokines, interleukins and proto-oncogenes (Caput et al. 1986; Shaw and Kamen 1986). Several ARE binding proteins (ARE-BPs) have been identified, which tightly regulate the turnover of transcripts they bind to. One of the ARE-BPs, having a stabilizing effect is the ELAV protein family member, HuR (Fan and Steitz 1998). In contrast, the CCCH tandem zing-finger protein tristetraprolin (TTP) promotes mRNA degradation

by recruiting decay enzymes targeting decapping, deadenylation and 5' to 3' exonucleolytic decay (Lai et al. 1999; Lykke-Andersen and Wagner 2005). Many 3' UTR regulatory sequences have been implicated in mRNA regulation in the germ line and will be reviewed together with trans-acting factors in section 2.3.

2.2.2.1. Trans-acting factors

Proteins that bind to 3' UTR elements can influence the fate of the mRNA in several ways. Many proteins have been shown to regulate mRNA transport by binding to specific sequences. Moreover, the assembly of repressive complexes can sequester mRNAs away from the translation machinery. Another common mechanism to interfere with translation is employed by 4E-BPs that disrupt the eIF4E-eIF4G interaction (Fig. 6).

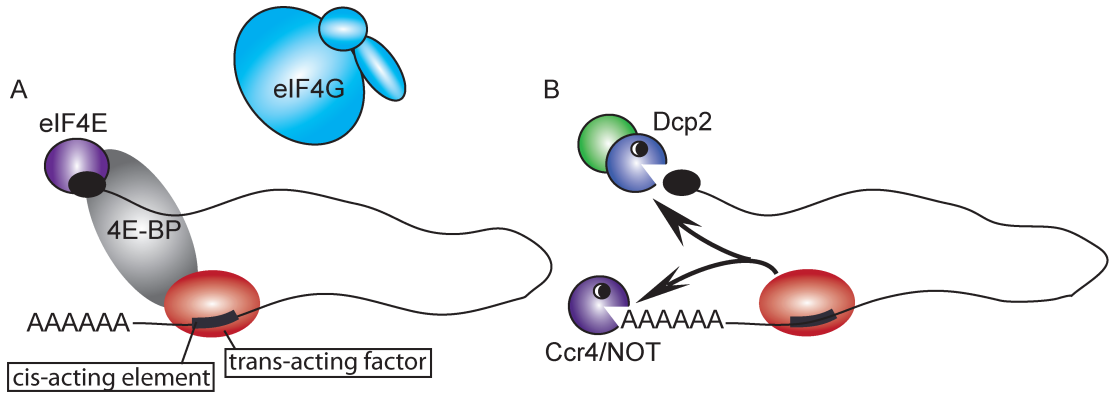

Figure 6
Sequence-specific mRNA repression. Cis-acting elements are bound by sequence specific trans-acting factors. (A) 4E-BPs interfere with the eIF4E-4G interaction by competing with eIF4G for eIF4E binding. This prevents the closed loop formation and translation initiation. (B) RBPs recruit mRNA decay enzymes to induce target mRNA degradation.

Alternatively, trans-acting factors can recruit mRNA decay enzymes and induce mRNA degradation. The different mechanisms will be discussed with the help of several examples in the context of maternal RNA regulation.

Other trans-acting factors besides RBPs are small RNAs like miRNAs and piRNAs. miRNAs constitute the most abundant class of small RNAs and are crucial for the regulation of virtually every cellular process. miRNAs are initially transcribed by RNA Pol II as long pri-miRNA precursors. Pri-miRNAs then fold into hairpins,

which are processed in two steps: firstly in the nucleus by the endoribonuclease Drosha into pre-miRNAs and secondly after their transport into the cytoplasm by Dicer into the mature ~22nt long miRNA. The mature miRNA is incorporated together with several RBPs into the miRNA induced silencing comlex (miRISC). The miRISC recognizes its targets via basepairing between the miRNA and the target 3' UTR and usually promotes target mRNA repression by either inducing translational repression or mRNA degradation. Key components of the miRISC, and crucial for target mRNA repression are the Argonaute and GW182 proteins, which interact with other proteins to affect translation initiation or recruit mRNA decay enzymes (Krol et al. 2010).

Another example of small RNAs is the class of 22 to 30 nt long piRNAs. Also piRNAs act on their targets with members of the Argonaute/Piwi protein family. piRNAs have not been extensively studied to date and so far are best known for their role in promoting genome stability in the germ line by transposon silencing. Accordingly, germ cell development is affected by mutations that disrupt the piRNA pathway (Khurana and Theurkauf 2010).

2.2.3. RNA granules

Importantly, mechanisms targeting an mRNA molecule are not executed by a single protein but are mediated by many factors that often concentrate in discrete cytoplasmic foci, known as RNA granules. RNA granules have been implicated in mRNA transport, translational repression, storage and decay.

2.2.3.1. Transport granules

If an mRNA needs to be localized to a specific cytoplasmic site it is packaged into transport particles that not only ensure the proper localization of the mRNA but also its translational repression during the transport. For example in budding yeast, localization of the *ASH1* mRNA ensures that the transcriptional repressor Ash1p is exclusively expressed in the daughter cell where it represses mating type switching

(Bobola et al. 1996; Long et al. 1997; Takizawa et al. 1997). Also in neurons mRNAs are actively transported to specific subcellular compartments in so-called neuronal granules as for example *β-actin* mRNA, which is thereby transported to the cell periphery, its sits of translation. The transport of the *β-actin* and the repression during its localization is mediated by the zipcode binding protein 1 (ZBP1), which binds RNA 3' UTRs through the zipcode element (Ross et al. 1997; Huttelmaier et al. 2005). The role of mRNA localization is probably most extensively studied and best understood during *Drosophila* oogenesis and early embryogenesis and will be discussed in this context below.

2.2.3.2. Processing bodies and stress granules

Another type of RNA granules are processing or P bodies, which were initially identified as sites of mRNA decay. Accordingly many decay factors such as Dcp2, Ccr4 and Xrn1 haven been shown to localize to P bodies (Sheth and Parker 2003). More recently, it was observed that several other pathways like NMD, ARE-mediated mRNA degradation, miRNA-induced repression and 4E-BP-mediated inhibition of translation initiation can take place in processing bodies. In contrast, P bodies seem to be devoid of ribosomes and translation factors with the exception of eIF4E (Andrei et al. 2005; Ferraiuolo et al. 2005; Liu et al. 2005; Pillai et al. 2005; Sen and Blau 2005; Sheth and Parker 2006; Franks and Lykke-Andersen 2007). This suggests that mRNAs within P bodies are not translated but are instead translationally repressed or degraded. The precise function of P bodies is however still not well understood since both mRNA repression and mRNA decay can occur in the absence of microscopically visible cytoplasmic foci, suggesting that P body formation is rather the consequence then the cause of mRNA regulation (Decker et al. 2007; Eulalio et al. 2007; Stalder and Muhlemann 2009). Under stress conditions, phosphorylated eIF2α induces the formation of granules closely related to P bodies, termed stress granules (Kedersha et al. 1999; Kedersha et al. 2005). Stress granules contain virtually all components of the 48s translation initiation complex and are therefore thought to incorporate mRNAs with stalled ribosomes derived from disassembled polysomes. Interestingly, P bodies and stress granules also physically associate with each other, suggesting that mRNAs

are first sorted within stress granules and if destined to undergo degradation are deposited into processing bodies (Kedersha et al. 2005).

2.2.3.3. Germ granules

Germ granules have been identified in essentially every organism investigated and include germinal granules in *Xenopus*, polar granules in *Drosophila*, and P granules in *C. elegans*. Germ granules contain mRNAs important for germ cell specification and proteins that function in RNA metabolism. Besides holding species-specific proteins like Oskar in *Drosophila* or PGL-1/2/3 in *C. elegans*, germ granules in different organisms also share some components like the germline helicases (*Xenopus* XVLG-1, *Drosophila* Vasa, *C. elegans* GLH-1/2/3/4) (Hay et al. 1988; Fujiwara et al. 1994; Breitwieser et al. 1996; Gruidl et al. 1996; Kawasaki et al. 1998). Mutations in germ granule components often not only disrupt RNA granules but also cause defects in germline development (Spike et al. 2008). Moreover, *Drosophila* pole plasm, which contains polar granules, can induce ectopic germ cell formation and the loss of germ cell identity in *C. elegans* is accompanied by the loss of P granules (Illmensee and Mahowald 1974; Ciosk et al. 2006). While germ granules have been implicated to regulate post-transcriptional gene expression and germline determination, their precise function remains unknown.

2.3. mRNA regulation during development

While mRNAs are regulated throughout the life cycle of an organism, I will only discuss events taking place during the OET, which is most relevant for this study. mRNA regulation during OET has been most extensively studied in mice, *Xenopus*, *Drosophila* and *C. elegans* and I will therefore focus on these four model organisms.
As previously mentioned, oocyte maturation and early embryogenesis take place in the absence of transcription, which means that these developmental stages depend solely on maternal factors that have been deposited in the oocyte during gametogenesis. Until maternal mRNAs are required during oogenesis or early

embryogenesis they need to be stabilized and translationally repressed, leading to maternal mRNA storage. The concept of mRNA storage has already been described as mRNA masking in 1966. RBPs bind mRNAs to mask them from the translation machinery and mRNA decay factors (Spirin 1966). While it is still unclear how and if mRNAs are protected from degradation, many examples of translational repression in the germ line have been described. At the mid-blastula transition (MBT), the embryo no longer depends on maternally contributed mRNAs but switches to zygotically encoded transcripts. Accordingly, the onset of zygotic gene activation (ZGA) occurs simultaneously with the degradation of maternal mRNAs.

2.3.1. Common players in mRNA regulation in the germ line

Besides species-specific proteins, a set of RBPs regulating germline mRNAs is also shared between species. Among these shared proteins is the DDX6 helicase (mammalian RCK/p54, *Xenopus* Xp54, *Drosophila* Me31B, *C. elegans* CGH-1, yeast Dhh1p), and its binding partner RAP55 (*Xenopus* RAP55, *Drosophila* TraI, *C. elegans* CAR-1, yeast Scd6). DDX6 helicases have been shown to localize to P bodies in yeast and humans were they activate mRNA decapping and repress translation (Coller et al. 2001; Sheth and Parker 2003; Cougot et al. 2004; Coller and Parker 2005). Similarly, DDX6 helicases localize to specific cytoplasmic foci in the germ line. These granules share some components with processing bodies but importantly seem to be devoid of RNA decay factors. This suggests that they might be sites of mRNA storage instead of mRNA decay and were therefore termed storage bodies in *C. elegans* (Flemr et al. 2010; Boag et al. 2008; Gallo et al. 2008; Lin et al. 2008; Noble et al. 2008).

While the DDX6 homologues in *Xenopus* and *Drosophila* were also shown to mediate translational repression of germline RNAs (Minshall et al. 2001; Nakamura et al. 2001), DDX6 helicases have recently been implicated in mRNA stabilization in Plasmodium and *C. elegans* (Mair et al. 2006; Boag et al. 2008). Interestingly, although exerting different mechanisms of mRNA regulation in different species, DDX6 helicases seem to do so by being part of a conserved germline protein complex that includes RAP55, PABP and Y-box proteins (Ladomery et al. 1997; Paynton

1998; Nakamura et al. 2001; Boag et al. 2005; Wilhelm et al. 2005; Pepling et al. 2007). Since DDX6 helicases are thought to possess little RNA binding specificity (Linder 2006), they are likely to be brought to target mRNAs via sequence specific RBPs (Fig. 7).

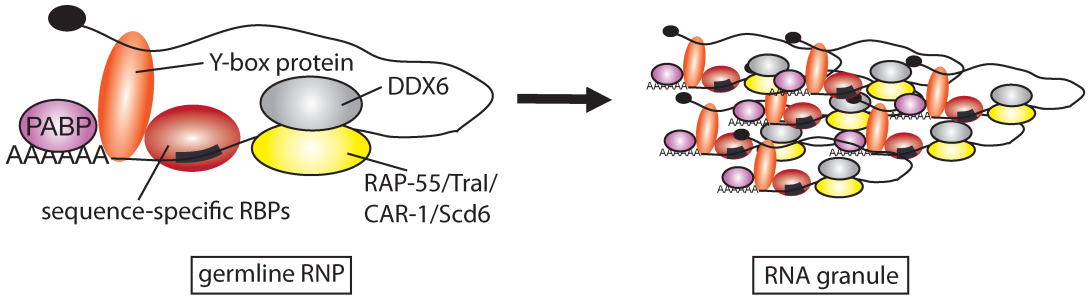

Figure 7
A set of conserved RBPs assembles into a conserved germline RNP. PABPs, DDX6 helicases, Y-box proteins and RAP-55 homologues associate with sequence specific RBPs on germline RNAs. Several of these germline RNPs aggregate into microscopically visible RNA granules.

Also the PUF (Pumilio and FBF) family of RBPs has been shown to play an important role in regulating post-transcriptional gene expression in the germ line in many species (Lin and Spradling 1997; Forbes and Lehmann 1998; Crittenden et al. 2002; Bachorik and Kimble 2005; Kadyrova et al. 2007). PUF proteins bind to a similar consensus motif, usually located in the 3' UTR of its targets (Zamore et al. 1997; Zhang et al. 1997). PUF proteins are generally known to mediate translational repression but can also stimulate mRNA deadenylation by recruiting the Ccr4-Not complex (Goldstrohm et al. 2006). Additionally, PUF proteins have been shown to target several translation initiation factors. In yeast, Puf6p binds and thereby inhibits eIF5B (Deng et al. 2008). In contrast, *Drosophila* Pum and *Xenopus* Pum2 interfere with translation initiation by targeting eIF4E, either via recruitment of the eIF4E competitor dE4HP or by competing with eIF4E for cap binding, respectively (Cao et al. 2010; Cho et al. 2006). More recently, PUF proteins have also been implicated in activating translation. For example in *C. elegans*, FBF not only induces the deadenylation of the *gld-1* transcript but also stimulates *gld-1* polyadenylation by GLD-2 (Suh et al. 2009).

Meiotic maturation in many organisms depends on the polyA polymerase GLD-2 that targets mRNAs for polyadenylation. In mice however, oocyte maturation does not seem to depend on GLD-2 (Nakanishi et al. 2006; Nakanishi et al. 2007). GLD-2 polyA polymerases differ from conventional eukaryotic polyA polymerases as they lack an RNA recognition motif (RRM) but are recruited to their targets via other RBPs (Wang et al. 2002).

2.3.2. Maternal mRNA regulation in vertebrates

Maternal mRNAs are often regulated via the length of their polyA tail. As previously discussed the polyA tail stimulates translation and although there are exceptions, polyadenylated mRNA can get translated while mRNAs with a short polyA tail are generally translationally repressed.

2.3.2.1. Translational repression of maternal mRNAs

The stage for polyA tail length regulation in *Xenopus* oocytes is already set in the nucleus, where the hexanucleotide sequence AAUAAA is recognized by the cytoplasmic polyadenylation specificity factor (CPSF). CPSF binds an mRNA with several other factors such as the scaffold protein symplekin. CPSF binding induces the cleavage of the pre-mRNA 20 – 30 nt downstream of the hexanucleotide and subsequently pre-mRNA polyadenylation in the nucleus. The polyadenylated mRNA is then exported into the cytoplasm, where it remains bound by symplekin and CPSF. In the cytoplasm a second 3' UTR element becomes crucial for the regulation of the polyA tail length, the cytoplasmic polyadenylation element (CPE). CPE-containing mRNAs get bound by the CPE binding protein (CPEB), which forms a complex with multiple proteins (Paris et al. 1991). In occytes, CPEB associates with the deadenylase PARN and the polyA polymerase GLD-2. Since the activity of PARN overrides the activity of GLD-2, this association leads to the shortening of the polyA tail of CPE mRNAs. Upon oocyte maturation, CPEB phosphorylation causes PARN

to get expelled from this complex. CPE mRNAs are then polyadenylated by GLD-2 and can get translated (Barnard et al. 2004; Kim and Richter 2006).
Additionally, CPEB binding prevents translation initiation by interacting with 4E-BPs. In early oocytes CPEB binds the DEAD box helicase Xp54 (mammalian RCK/p54), the RBPs P100 (mammalian Pat1a/b) and RAP55 (mammalian RAP55), eIF4E and the 4E-BP eIF4E-Transporter (4E-T). Tethering of 4E-T leads to mRNA repression in a cap-dependent manner. Although 4E-T is a 4E-BP, it does not prevent translation by competing with eIF4G binding, but instead binds the alternative eIF4E, eIF4E1b, which has little binding affinity for the cap and eIF4G (Minshall et al. 2007). In later stages of *Xenopus* oogenesis, another 4E-BP, Maskin, seems to prevent translation via the canonical 4E-BP pathway by competing with eIF4G for eIF4E binding (Stebbins-Boaz et al. 1999). Upon oocyte maturation Maskin gets phorphorylated, which leads to its dissociation from the eIF4E, thus allowing the translation of CPE-containing mRNAs during embryogenesis (Barnard et al. 2005).

The regulation of the polyA tail length also plays a central role in murine oogenesis. mRNAs are known to undergo polyadenylation during oocyte maturation (Huarte et al. 1987; Vassalli et al. 1989; Gebauer et al. 1994; Racki and Richter 2006). Furthermore, the factors influencing the polyA status of mRNAs seem to be similar as CPEB mediates mRNA polyadenylation and oocyte maturation not only in *Xenopus* but also in mice (Hodgman et al. 2001; Racki and Richter 2006). For example, CPEB promotes the polyadenylation and translation of the *Dazl* mRNA. DAZL itself is essential for oocyte maturation and early embryonic development and can, once translated, activate the translation of its target mRNAs (Chen et al. 2011).

2.3.2.2. Stabilization of maternal mRNAs

In vertebrates no designated mechanism mediating maternal mRNA stability is known. It seems however that at least in *Xenopus*, mRNAs are protected from degradation because mRNA decay is generally inhibited. The deadenylation activity is slow in oocytes and increases only upon fertilization. Furthermore, no decapping activity could be detected when extracts from *Xenopus* oocytes and early embryos

were analyzed. These findings also explain why deadenylated transcripts are not degraded via the canonical 5' to 3' decay pathway and are unusually stable until the MBT (Voeltz and Steitz 1998; Zhang et al. 1999). Furthermore, the activity of miRNAs seems to be repressed during mouse oogenesis (Ma et al. 2010; Suh et al. 2010). Since miRNA-mediated mRNA repression often leads to target mRNA degradation, the inhibition of miRNAs might be important for maternal mRNA storage and the oocyte-to-embryo transition.

2.3.2.3. Reactivation and degradation of maternal mRNAs

Upon egg activation several events ensure that maternal mRNAs in *Xenopus* are reactivated. The deadenylase PARN is expelled from repressive ribonucleoprotein (RNP) complexes, enabling GLD-2 to polyadenylate mRNAs required for meiotic maturation like *mos* and *cycB1*. At the same time the block of translation initiation is relieved by the dissociation of Maskin, thereby allowing these mRNAs to get translated (Sheets et al. 1995; Stebbins-Boaz et al. 1996).
The mechanisms responsible for the degradation of maternal mRNAs are not well understood but, at least in zebrafish, miRNAs have been shown to mediate the clearance of maternal mRNAs during early embryogenesis (Giraldez et al. 2006).

2.3.3. Translational regulation in *Drosophila* development

In the *Drosophila* oocyte and embryo the major body axes are established by the localized translation of cell fate determinants such as *oskar*, *bicoid* and *gurken*. mRNAs in early *Drosophila* development are generally transcribed in nurse cells surrounding the oocyte and get transported to the sites where they are needed. 71% of the mRNAs in *Drosophila* embryos show a specific subcellular localization, which can be attained in several ways (Lecuyer et al. 2007). Transcripts can either be locally protected from degradation or they are passively or actively transported to the site where they are needed. Many mRNAs in *Drosophila* oocytes are actively transported but until they reach their destination they need to be translationally repressed. The

combination of restricted translation and translational repression during mRNA transport creates protein gradients of cell fate components.

2.3.3.1. Translational repression of maternal mRNAs

The mechanisms operating to regulate mRNAs in *Drosophila* oocytes are somewhat similar to the ones identified in *Xenopus*. Also in *Drosophila*, a 4E-BP protein has been reported to repress translation initiation by disrupting the eIF4E-eIF4G interaction (Nakamura et al. 2004): Cup is recruited to its target mRNAs via RBPs that recognize specific 3' UTR sequences and Cup-mediated translational repression is crucial during early *Drosophila* development.
Additionally, the polyA tail length of many fly mRNAs has been shown to influence the translation of these mRNAs. For example, the translation of several cell fate determinant transcripts coincides with the lengthening of their polyA tail during embryogenesis and at least *bicoid* translation depends on polyadenylation (Salles et al. 1994). Conversely, Nanos-mediated repression of *hunchback* is mediated by promoting *hunchback* deadenylation (Wreden et al. 1997). However, the relationship between the polyA tail length and the translational status of an mRNA is not that simple and *nanos* translational repression for example can occur independently of deadenylation (Salles et al. 1994).

The translation of several mRNAs is tightly regulated during *Drosophila* oogenesis and is mediated by various RBPs. For instance, Squid binds the *gurken* mRNA already in nurse cells and promotes together with Cup and PABP *gurken* translational repression during transport. When *gurken* reaches the dorsal-anterior region of the oocyte the repressive RNP complex is remodeled and Encore (Enc) together with PABP induces the translational activation of *gurken* (Clouse et al. 2008; Caceres and Nilson 2009).

Another well-studied example of translational regulation in *Drosophila* oocytes is the *oskar* mRNA. The encoded protein, Oskar, directs posterior patterning and germ cell formation in the early embryo and, if mis-expressed, induces ectopic germ cell

formation (Lehmann and Nusslein-Volhard 1986; Ephrussi and Lehmann 1992). To restrict Oskar protein expression, *oskar* is transported from its site of transcription in nurse cells to the posterior pole of the oocyte (Ephrussi et al. 1991). During this transport *oskar* translation is prevented by the assembly of repressive RNP complexes. The assembly of these RNPs begins already in the nucleus with the deposition of EJCs during splicing, which allows *oskar* to assemble into multi-mRNP particles (Ephrussi and Lehmann 1992; Kim-Ha et al. 1995; Hachet and Ephrussi 2004). During the transport of these RNPs to their destination, *oskar* translation is prevented by Bruno, Hrp48 and PTB binding to specific sequences located within the UTR of *oskar* (Kim-Ha et al. 1995; Yano et al. 2004; Besse et al. 2009). Bruno-mediated translational repression of *oskar* involves at least two distinct mechanisms. On one hand, Bruno inhibits translation initiation in a cap-dependent way, by recruiting the 4E-BP Cup. In a second cap-independent mode of inhibiting translation initiation, Bruno induces the formation of *oskar* "silencing particles", which are inaccessible to the translation machinery (Chekulaeva et al. 2006). Interestingly, the *oskar* mRNA itself seems to have a function as a scaffold factor during early *Drosophila* oogenesis as well (Jenny et al. 2006).

The *nanos* mRNA encodes for the posterior cell fate determinant Nanos (Wang and Lehmann 1991) and is repressed until it reaches the posterior pole by the RBPs Glorund (Glo) and Smaug (Smg). Both proteins bind to stem loops within the 3' UTR of *nanos* thus influencing the fate of the message. Glorund prevents *nanos* translation in oocytes by interfering with translation initiation and targeting translation at a post-initiation step. The Glorund mediated repression at the post-initiation level persists until Smaug takes over *nanos* translation during embryogenesis (Andrews et al. 2011; Smibert et al. 1996; Kalifa et al. 2006). Consistent with *nanos* being repressed at a post-initiation step are the observations that *nanos* repression can occur cap-independently on ribosomes via the nascent polypeptide associated complex (Jeske et al. 2011; Markesich et al. 2000).

In the embryo, Smaug induces *nanos* mRNA degradation in the anterior embryo by recruiting the deadenylase CCR4-NOT (Zaessinger et al. 2006). Additionally, Smaug recruits the 4E-BP Cup, Me31B (RCK/p54) and TraI (RAP-55) which blocks the eIF4E-eIF4G interaction. The formation of this repressive complex *in vitro* leads to

eIF4G displacement and prevents 48s complex formation, which is in agreement with only a minor fraction of *nos* being associated with polysomes (Jeske et al. 2011; Nelson et al. 2004; Qin et al. 2007). At the posterior, *nanos* translational repression and degradation is relieved by Oskar, which competes with Smaug for *nanos* binding.

2.3.3.2. Stabilization of maternal mRNAs

The mechanisms mediating mRNA stabilization in *Drosophila* are not well understood. However, in contrast to *Xenopus*, mRNA decay pathways seem to be active in *Drosophila* oogenesis. The *bicoid* mRNA is specifically stabilized by the bicoid specificity factor (BSF) binding to the *bicoid* 3' UTR (Mancebo et al. 2001). This suggests that a designated mechanism to prevent precocious maternal mRNA degradation has to exist.

2.3.3.3. Reactivation and degradation of maternal mRNAs

The reactivation of maternal mRNAs in *Drosophila* is mediated consecutively by two polymerases. The *Drosophila* CEPB homologue Orb associates with a certain polyA polymerase in early oocytes, whereas it binds GLD-2 during late oogenesis and early embryogenesis (Benoit et al. 2008). GLD-2 is not only necessary for egg activation but also for maternal mRNA destabilization, probably by activating the translation of genes important for oogenesis and egg activation via polyadenylation (Tadros et al. 2003; Cui et al. 2008).

Upon egg activation maternal mRNAs are degraded in *Drosophila* in two waves. The first wave is mediated by maternally encoded factors and is triggered by egg activation whereas the second wave becomes active with the onset of zygotic transcription. The first wave is regulated by the miR309 cluster and since Smaug is crucial for the expression of these miRNAs, *smaug* mutants are defective for maternal mRNA degradation. Interestingly, also the onset of zygotic transcription requires Smaug, suggesting that maternal mRNAs need to be degraded before zygotic transcription can be activated (Thomsen et al. 2010; Bashirullah et al. 1999; Tadros et

al. 2007; Bushati et al. 2008; Benoit et al. 2009). Also piRNAs have been linked to maternal mRNA degradation in *Drosophila*. Smaug binds the *nanos* 3'-UTR together with piRNAs, the Argonautes Aubergine (Aub) and Ago3, and the CCR-4 deadenylase thereby promoting *nanos* mRNA deadenylation and decay (Rouget et al. 2010).

Recent studies have begun to address how the interplay between maternal mRNA degradation and zygotic transcription contribute to the total mRNA level in the embryo (Thomsen et al. 2010; De Renzis et al. 2007).

2.4. *C. elegans* development

Also *C. elegans* development relies heavily on translational regulation, mediated by many RBPs. Especially in the *C. elegans* germ line, many developmental decisions are regulated at the post-transcriptional level. A recent study on germline expressed RNAs revealed, that the translational machinery is strongly enriched in the germ line compared to the soma, supporting the notion that post-transcriptional gene regulation is very important in the germ line (Wang et al. 2009). Consistently, the expression pattern of most germline proteins can be mimicked by 3' UTR fusions but not promoter fusions, showing that post-transcriptional regulation rather than transcriptional regulation is the main contributor to the control of gene expression in the germ line (Merritt et al. 2008).

2.4.1. The *C. elegans* germ line

Caenorhabditis elegans is a 1 mm long transparent nematode, which in recent decades, proved to be a versatile model organism to study numerous biological processes. The *C. elegans* life cycle comprises embryogenesis, four larval stages (L1 – L4) and adulthood. *C. elegans* has two sexes: males and hermaphrodites. Whereas males continuously produce sperm in their life, germ cells in the hermophrodites differentiate into sperm during the last larval stage and develop into oocytes throughout adulthood. Sperm in hermaphrodites is stored in the spermatheca until needed, thus allowing a *C. elegans* hermaphrodite to reproduce by self-fertilization. A

C. elegans hermaphrodite contains two U-shaped gonad arms, where germ cells develop in a linear fashion as they move from the distal to the proximal end: germ cells in the adult gonad initially divide mitotically in the distal region, they then enter meiosis and finally undergo oogenesis in the proximal gonad arm (Fig. 8).

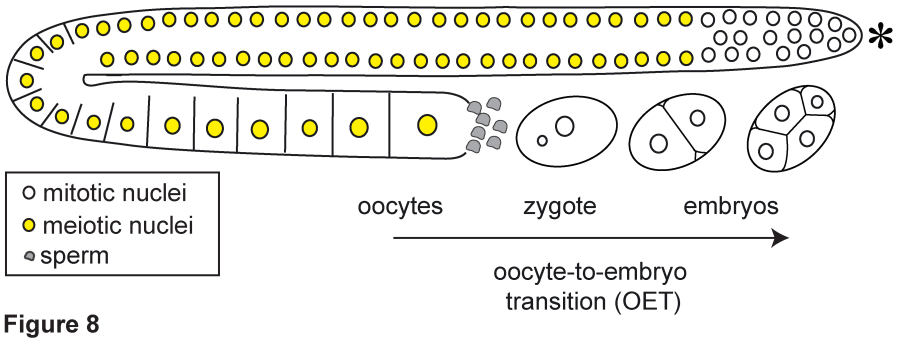

Figure 8
Depicted is one of the two U-shaped gonad arm of an adult *C. elegans* hermaphrodite. The distal end of the germ line is indicated with an asterisk. Cells initially divide mitotically and as they move proximally they enter meiosis and progress through meiotic prophase. Differentiated oocytes in the proximal gonad arms get self-fertilized and start embryogenesis in the uterus.

The somatic distal tip cell (DTC) is located at the tip of the mitotic region and generates a stem cell niche by promoting mitotic proliferation of undifferentiated germ cells (Kimble and White 1981). The mitotic proliferation is driven by Notch signaling (Austin and Kimble 1987), which is restricted to the distal gonad by the proximity of germ cells to the DTC, expressing the Notch ligand LAG-2 (Henderson et al. 1994; Crittenden et al. 2006). Additionally, the expression of the Notch receptor, GLP-1, is limited to distal germ cells, further confining mitotic proliferation to the distal region of the gonad (Crittenden et al. 1994). Notch signaling not only stimulates mitotic proliferation but also inhibits meiotic entry. When germ cells move distally they are no longer exposed to Notch signaling and enter into meiosis.

Entry into meiosis is promoted by two parallel pathways. On one hand, the Bicaudal-C homologue GLD-3 pairs with the polyA polymerases GLD-2 and GLD-4 to activate the translation of meiosis-promoting mRNAs, including *gld-1* (Eckmann et al. 2002; Wang et al. 2002; Eckmann et al. 2004; Suh et al. 2006; Schmid et al. 2009). In the second pathway, the Nanos protein NOS-3 activates the translational repressor GLD-1, which then prevents the expression of mitosis promoting genes like *glp-1*, cyclin E (*cye-1*) and *fbf-1/2* (Marin and Evans 2003; Hansen et al. 2004; Kimble and

Crittenden 2007; Biedermann et al. 2009). Germ cells exit mitosis and enter meiosis in the transition zone: chromosomes start to pair and undergo homologous recombination. Germ cells have completed the pachytene stage when they reach the loop region of the gonad arm. Up to this point *C. elegans* germ cells have developed in a syncytium. In the loop regions, the enclosure of germ cells with a plasma membrane occurs at the same time as the progression from pachytene through diplotene, which is mediated by the mitogen-activated protein kinase (MAPK) (Church et al. 1995).

In the proximal gonad arm, the chromosomes of the developing oocytes get highly condensed into homologous pairs during diakineses. Oocyte ovulation is mediated by the major sperm protein (MSP). On one hand, MSP promotes oocyte maturation by activating the MAPK signaling cascade in oocytes. And secondly, MSP stimulates the contraction of smooth muscle-like gonadal sheath cells (Miller et al. 2001). The most proximal oocyte then enters the spermatheca and gets fertilized. During early embryonic development, eight asymmetric cell divisions produce 16 founder cells that give rise to all body lineages.

2.4.2. Translational regulation in the *C. elegans* germ line

As previously mentioned, the *C. elegans* germ line relies heavily on post-transcriptional gene regulation. RBPs regulate many developmental decisions in the germ line and often function in more than one developmental decision (Fig. 9).

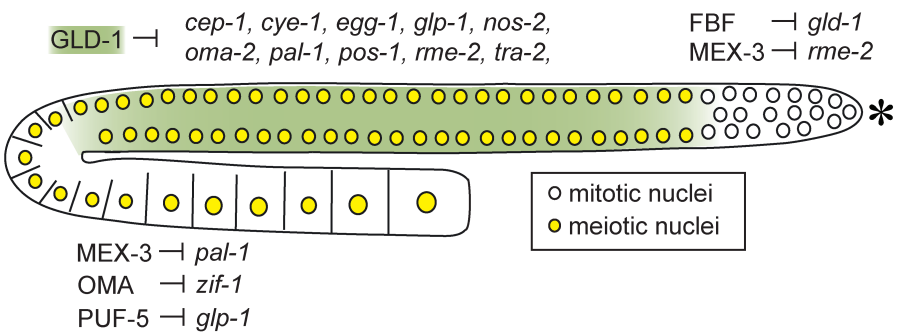

Figure 9
Several translational repressors including some of their target mRNAs are depicted where they act in the gonad.

2.4.2.1. Translational regulation in germline stem cells

In the distal region of the gonad, the Pumilio RBPs FBF-1/-2 promote germline stem cell maintenance by preventing precocious entry into meiosis. In part this is achieved by FBF-1/2 preventing the expression of synaptonemal complex proteins and activators of meiotic entry, GLD-1 and GLD-3 (Merritt and Seydoux 2010; Crittenden et al. 2002; Eckmann et al. 2004). FBF-1/2 activity is itself regulated in different ways. The expression of at least FBF-2 is activated by Notch signaling and is restricted to the distal region by a negative autoregulatory feedback loop. Additionally, GLD-3 can antagonize the activity of FBF-1/2 (Eckmann et al. 2002; Lamont et al. 2004). Another RBP that functions in germ line stem cells is the KH domain protein MEX-3. Together with *C. elegans* ataxin 2, ATX-2, MEX-3 prevents the translation of the mRNA encoding for the yolk receptor protein RME-2. ATX-2 not only influences MEX-3 mediated translational regulation but also has an effect of GLD-1 dependent mRNA repression in the central gonad (Ciosk et al. 2004).

2.4.2.2. Translational regulation in the central gonad

GLD-1 stands for “defective in germ line development” and is as its name suggests a key regulator in the *C. elegans* germ line. Besides mediating meiotic entry, GLD-1 is also critical for meiotic progression, the maintenance of germ cell identity, sex determination and gametogenesis (Fig. 10).

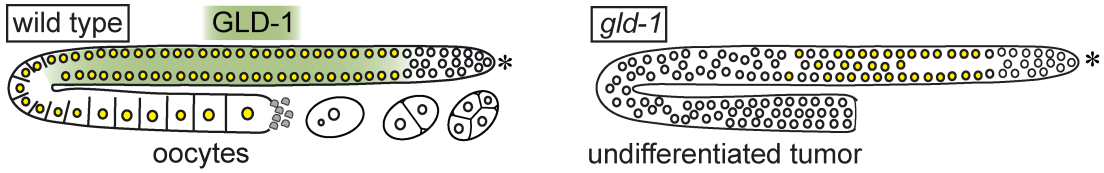

Figure 10
Germ cells in a wildtype gonad enter meiosis and differentiate into oocytes in the proximal gonad arm. In contrast, germ cells in the *gld-1* mutant enter but fail to progress through meiosis. Instead they re-enter into the mitotic cell cycle and form a proliferative tumor in the proximal gonad arm.

Germ cells in *gld-1* null mutants exit the mitotic cell cycle but fail to progress through meiosis, leading to the formation of a proliferative tumor in the proximal gonad arm (Francis et al. 1995). Additionally, the loss of GLD-1 mediated repression of *cye-1*

(*C. elegans* cyclin E) induces somatic differentiation in the central germ line, an appearance reminiscent of human germ cell tumors termed teratoma (Ciosk et al. 2006; Biedermann et al. 2009). GLD-1 is a member of the STAR (signal transduction and activation of RNA) family of RBPs, which will be discussed in more detail below, and is known to function as a translational repressor of various mRNAs in the central germ line (Jan et al. 1999; Lee and Schedl 2001; Marin and Evans 2003; Mootz et al. 2004; Schumacher et al. 2005; Biedermann et al. 2009). Recently, more then 900 germline mRNAs have been shown to associate with GLD-1 (Wright et al. 2010). The importance of the RNA binding activity of GLD-1 is further supported by the fact that mutations in the KH RNA binding domain induce a phenotype similar to the *gld-1* null mutant (Jones and Schedl 1995).

2.4.2.3. Translational regulation in oocytes

Two already introduced players, FBF-1/2 and MEX-3, repress the translation of transcripts not only in germline stem cells but also in other regions of the gonad. In oocytes GLS-1 releases FBF-1/2 from GLD-3 mediated repression, and FBF-1/2 can then stimulate oogenesis by inhibiting the translation of sperm-promoting genes like fog-1 and fem-3 (Zhang et al. 1997; Thompson et al. 2005; Rybarska et al. 2009). MEX-3 prevents the expression of the embryonic cell fate determinant PAL-1 in oocytes (Mootz et al. 2004). Interestingly, while the KH-domain protein is known to repress the translation of *rme-2* in the distal gonad, it is unable to do so in oocytes and instead allows *rme-2* translation. Besides FBF-1/2, other Pumilio proteins function in translational regulation in oocytes, where the translation of *glp-1* is prevented by PUF-5/6/7 (Lublin and Evans 2007). Additional RNA regulators in oocytes are the two redundantly functioning CCCH zinc finger proteins OMA-1 and OMA-2. OMA-1 represses the translation of *zif-1* in oocytes by recruiting the 4E-BP SPN-2. Upon fertilization, OMA-1 gets phosphorylated, which leads to the exclusion of SPN-2 from the complex and *zif-1* translation (Guven-Ozkan et al. 2010). ZIF-1, which is a subunit of the E3 ubiquitin ligase, can then function in the embryo to mediate cullin-dependent degradation of germline proteins in somatic blastomeres and thus promotes germline establishment (DeRenzo et al. 2003).

2.4.2.4. Mechanisms of translational regulation

While many translational regulators have been studied in the *C. elegans* germ line, very little is known about how translational regulation is mediated. It seems however that also in *C. elegans* regulators of the polyA tail length and 4E-BPs are implicated in translational regulation. As already mentioned OMA-1 represses *zif-1* translation by associating with 4E-BP SPN-2 and FBF-1/2 seem to repress *gld-1* by recruiting the CCR-4 deadenylase (Guven-Ozkan et al. 2010; Schmid et al. 2009). The repression of *gld-1* is alleviated by the two polyA polymerases GLD-4/GLS-1 and GLD-2/GLD-3 (Schmid et al. 2009). Besides regulating meiotic entry, GLD-2 also promotes the progression of germ cells through meiosis and influences the sperm/oocyte decision. GLD-2 forms a complex with the Bicaudal-C protein GLD-3 to promote spermatogenesis while it associates with RNP-8 to specify oogenesis (Kim et al. 2009). The polyA polymerase activity of GLD-2/RNP-8 is critical for oogenesis and ensures the polyadenylation and stabilization of many maternal mRNAs (Kim et al. 2010).

2.4.3. Early embryogenesis

After fertilization, transcription remains shut off until the 4-cell stage. This block of transcription is initially mediated by OMA-1 by sequestering a crucial component of the RNA Pol II initiation complex, TAF-4, in the cytoplasm (Guven-Ozkan et al. 2008). Interestingly, the same phosphorylation event that triggers the relief of OMA-1 mediated translation repression is also crucial for OMA-1 dependent inhibition of transcription and additionally promotes OMA-1 degradation (Nishi and Lin 2005). While OMA-1 only prevents transcription in the one-cell stage, PIE-1 ensures transcriptional repression until the 4-cell stage, in germ line blastomeres even until the 100-cell stage. PIE-1 represses transcription by mimicking a non-phosphorylatable CTD of RNA Pol II and competes with the CTD for binding to the transcriptional activator P-TEFb (Mello et al. 1996; Seydoux et al. 1996; Seydoux and Dunn 1997;

Batchelder et al. 1999; Zhang et al. 2003; Guven-Ozkan et al. 2008). Simultaneously with the onset of zygotic transcription at the 4-cell stage, class II maternal mRNAs start to get degraded in somatic blastomeres, while class I maternal mRNAs remain ubiquitously expressed (Seydoux and Fire 1994; Baugh et al. 2003). The mechanisms targeting maternal mRNAs for degradation have yet to be determined.

2.4.4. GLD-1 and other STAR proteins

As previously mentioned, GLD-1 is a member of the STAR protein family, which is characterized by the STAR domain consisting of a maxi-KH RNA binding domain, flanked by two Qua (Quaking) domain. While the first Qua domain mediates dimerization of STAR proteins, the second Qua domain facilitates RNA binding of the KH domain (Chen et al. 1997). STAR proteins have been shown to regulate a variety of developmental processes. More specifically, family members of the quaking-related (QR) subfamily of STAR proteins are important for tumor suppression by inhibiting cell proliferation and promoting differentiation (Biedermann et al. 2010). QR proteins include mammalian Quaking (QKI), *Drosophila* HOW and *C. elegans* GLD-1. The *qkI* locus gives rise to several QKI isoforms by alternative splicing, with QKI-5, QKI-6 and QKI-7 having been characterized in more detail (Hardy et al. 1996; Kondo et al. 1999). While *quaking* null mutant mice die between embryonic day 9.5 and 10.5, *quaking*^{viable} (*qk*^{v}) mutants develop tremors due to a hypomyelination in the central and peripheral nervous system (Sidman et al. 1964; Li et al. 2003). QKI-5 is expressed throughout embryogenesis whereas QKI-6/7 are only upregulated at the onset of myelination. Oligodendrocytes and Schwann cells are the myelinating cells of the central and peripheral nervous system respectively. In myelinating cells of *qk*^{v} mutant, the expression of the nuclear isoform QKI-5 is not affected but both cytoplasmic isoforms, QKI-6 as well as QKI-7, are absent in these mice (Ebersole et al. 1996; Wu et al. 1999). Since QKI-6/7 are required for the export of the mRNA encoding for the myelin basic protein (MBP) in oligodendrocytes, *qk*^{v} mutants show reduced MBP expression (Li et al. 2000; Larocque et al. 2002). Additionally, QKI-6/7 promote cell cycle arrest and oligodendrocyte differentiation by protecting the p27^{Kip1} mRNA and

thus ensuring protein expression of the cyclin-dependent kinase $p27^{Kip1}$. Recently QKI isoforms were also shown to induce Schwann cell differentiation by promoting the expression of MBP, $p27^{Kip1}$ and Krox-20, a transcription factor essential for PNS myelination (Larocque et al. 2009). Also the nuclear isoform QKI-5 has been implicated in mRNA regulation and was shown to regulate alternative splicing of the myelin-associated glycoprotein (MAG) (Wu et al. 2002). Hence the hypomyelination phenotype of qk^{v} mutants can be attributed to QKI isoforms regulating splicing, export and stability of various mRNAs important for glial cell differentiation. Interestingly, although mammalian QKI has so far not been shown to regulate mRNA translation, the QKI-6 isoform can mediate translational repression in *C. elegans* (Saccomanno et al. 1999).

Also the *Drosophila* STAR protein, HOW, comes in two isoforms that can exert different mechanisms of mRNA regulation. For example, while the longer, nuclear isoform HOW(L) induces the degradation of the *stripe* mRNA, cytoplasmic HOW(S) can counteract this activity and instead promotes the *stripe* stabilization and thereby tendon cell differentiation (Nabel-Rosen et al. 2002; Edenfeld et al. 2006). Other functions of HOW include the regulation of alternative splicing during glial cell maturation and mRNA repression during mesoderm development (Nabel-Rosen et al. 2005; Edenfeld et al. 2006; Toledano-Katchalski et al. 2007).
C. elegans has two STAR proteins: GLD-1, which so far has only been implicated in repressing mRNA translation and ASD-2, which regulates alternative splicing during development (Ohno et al. 2008).

Since the STAR domain is highly conserved amongst different species, not surprisingly also the RNA consensus sequence of various STAR proteins is very similar. Mammalian QKI binds its targets through the RBP recognition element (RRE) AYUAAY (Y being one of the pyrimidines C or U). Orginally, the motif was identified by systematic evolution of ligands by exponential enrichment (SELEX) and more recently was further defined by PAR-CLIP (Hafner et al. 2010; Galarneau and Richard 2005). Interestingly, the branch point sequence YNCURAY (N being any nucleotide, R being one of the purines A or G), mediating intron recognition, resembles the QKI RRE and is recognized by another member of the STAR protein

family, the mammalian intronic branch-site RBP SF-1 (Peled-Zehavi et al. 2001). In *C. elegans*, introns lack the branch point sequence but instead contain the consensus sequence UUUCAG/R at their 3' end (Aroian et al. 1993). While this motif does not match the sequence criteria of the GLD-1 binding motif (GBM), it is currently unclear if the second *C. elegans* STAR protein, ASD-2 associates with the 3' splice site. The recently identified GBM describes a degenerate 7-mer RNA motif that is related to the previously published STAR-binding element (Wright et al. 2010; Ryder et al. 2004). The GBM refers to 38 heptanucleotide sequences, which differ in their nucleotide composition and therefore affinity for GLD-1. Remarkably, the binding affinity of GLD-1 for each mRNA can be quantitatively predicted by considering the number and strength of individual GBMs within UTRs (Wright et al. 2010). The similarity between the RNA consensus sequences of different STAR proteins is further confirmed by the observation that mammalian QKI-6 is able to bind and also translationally repress at least one target of *C. elegans* GLD-1 (Saccomanno et al. 1999).

While the translational repressor GLD-1 has been extensively studied in the *C. elegans* germ line it is still largely unknown how GLD-1 represses its targets. The TGE element within the *tra-2* 3' UTR not only induces GLD–1 mediated repression and but also deadenylation. Interestingly, the TGE can also promote polyA tail-dependent translational repression in *Xenopus* embryos (Thompson et al. 2000). Additionally, loss of the TGE causes the *tra-2* message to be increasingly associated with polysomes, suggesting that TGE-mediated repression involves an inhibition of translation initiation (Goodwin et al. 1993). In contrast, GLD-1 has been suggested to interfere with translational elongation of *pal-1* (Mootz et al. 2004). Yet another study suggested that GLD-1 binding to two of its targets prevents the nonsense-mediated decay machinery from degrading these mRNAs (Lee and Schedl 2004). A more extensive analysis of GLD-1 mediated translational repression is however needed to gain insight into how GLD-1 might function.

2.5. Scope of this thesis

At the start of my thesis, it was known that various RBPs regulate the many developmental decisions in the *C. elegans* germ line. It had been discovered that the STAR-domain protein GLD-1 plays a role in many of these decisions such as promoting meiotic entry and progression, spermatogenesis and maintenance of germ cell identity. The translational repression of several mRNAs had been found to depend in GLD-1 and loss of the RNA binding activity of GLD-1 had been reported to phenocopy a null mutant phenotype. In contrast, the scale of translational repression and also the mechanism of GLD-1 mediated mRNA repression were unclear.

Addressing these questions, my thesis was aimed at understanding how GLD-1 globally regulates its target mRNAs. Towards this goal, we compared the polysomal association of wildtype and *gld-1* mutant worms. Interestingly we revealed a second function of GLD-1 in maternal mRNA protection.

Furthermore we wanted to identify GLD-1 interacting proteins to gain mechanistic insight into GLD-1-mediated RNA regulation. One of the interacting partners, CGH-1, had at that point been implicated in maternal mRNA protection, like GLD-1. This prompted us to further analyze the relation between GLD-1 and CGH-1 and their role in maternal mRNA regulation.

3. Results

3.1. The germ line is the main compartment of translational repression in *C. elegans*

3.1.1. Introduction

The importance of mRNA regulation in the *C. elegans* germ line has been extensively reviewed in section 2.2. Many *C. elegans* RBPs have been identified and were shown to regulate mRNA translation in the germ line. Accordingly, the 3'-UTR of several mRNAs is sufficient to recapitulate the protein expression pattern while investigated promoters only have a minor contribution to gene regulation. This suggests that in the germ line protein expression patterns are established via post-transcriptional regulation of gene expression (Merritt et al. 2008). Additionally, transcripts encoding for RNA regulators are greater than 4 fold more abundant in the germ line compared to the soma (Wang et al. 2009) and at least in *Drosophila* several RBPs seem to get post-transcriptionally downregulated after fertilization (Gouw et al. 2009).
The scale of mRNA repression in the *C. elegans* germ line is however still unknown. We globally investigated the ribosomal association of mRNAs in wildtype and germline-less worms by polysome profiling followed by tiling array analysis. The comparison between both worm strains allowed us to discriminate between mRNA repression in the germ line and the soma. We found that the germ line is the main compartment of translational repression in *C. elegans* but that mRNAs encoding for the translational machinery are specifically repressed in the soma.

3.1.2. Results

To examine the degree of mRNA repression in the germ line and in the soma, we analyzed worms with and without a germ line (wild type vs. *glp-4(bn2)* mutant worms) by polysome profiling. To separate mRNP complexes according to their size, whole worm extracts were fractionated by sucrose density gradient ultracentrifugation (Fig. 1). mRNAs present in light sucrose fractions are not associated with polysomes but are repressed – either because translation initiation is directly prevented or

because the mRNA is sequestered away from translation machinery. In contrast, actively translated mRNAs are present in heavier polysomal fractions. In addition, these fractions contain mRNAs that are part of heavy repressive mRNPs and mRNAs that are repressed during the translation elongation or termination step.

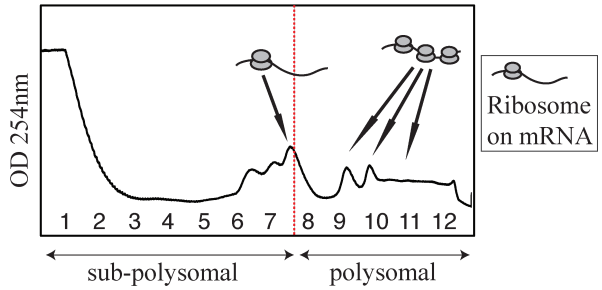

Figure 1
Typical polysome profile of wildtype worms. Positions of the 80s monosome and polysomes are indicated.

Polysomal RNA (fractions 8 to 12) and total RNA (fractions 1 to 12) from both wildtype and *glp-4* mutant gradients were Trizol extracted and globally analyzed by tiling arrays (Fig. 2). In the following scatter plots each dot represents a single transcript. mRNAs that are largely associated with polysomal fractions are represented as dots that lie on the diagonal of the scatter plot. Conversely, mRNAs that are at least to a certain extent associated with sub-polysomal fractions are represented as dots below the diagonal. Transcripts that are strongly enriched in sub-polysomal fractions, i. e. poorly translated mRNAs (polysomal mRNA/total mRNA < -1.5) are colored in red. While in wildtype worms, more than 835 mRNAs are strongly enriched in sub-polysomal fractions, only 59 mRNAs are poorly translated in germline-less animals.

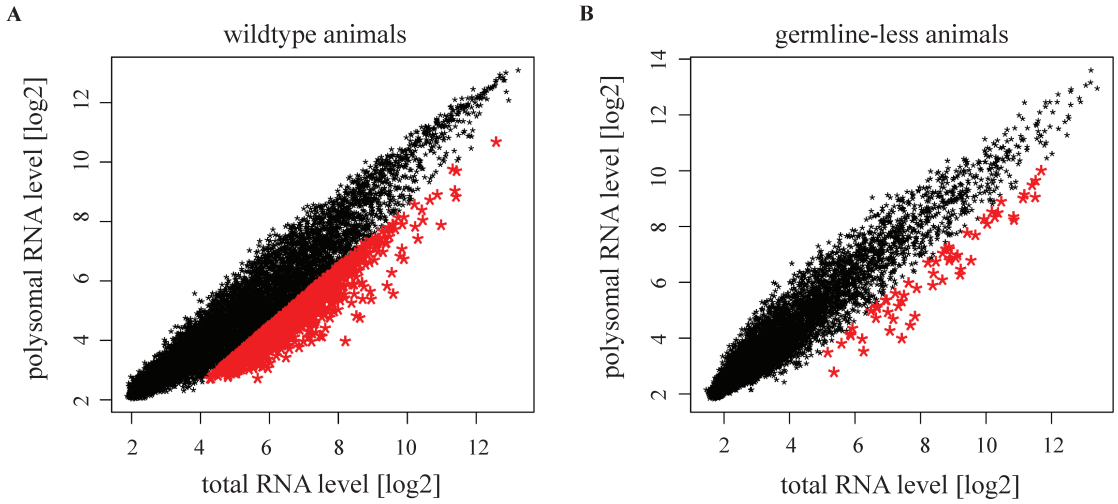

Figure 2
mRNAs that are poorly translated are mainly present in the germ line of *C. elegans*.
One dot in this and the following scatter plots represents a single transcript. Poorly translated mRNAs are colored in red and are defined as mRNAs with a polysomal/total mRNA ration < -1.5 (log2 scale) (A) 835 mRNAs are poorly translated in wildtype worms. (B). 59 mRNAs are poorly translated in germline-less mutant worms.

Poorly translated mRNAs can either be present in sub-polysomal fractions because they are generally not efficiently translated, for example because the start codon is located in a "non-optimal" context for translation initiation. However, the more likely alternative is that these mRNAs are poorly translated because a specific mechanism is targeting them for repression.

Since the mRNA repression observed in germline-less animals corresponds to mRNA repression in the soma, the big difference in mRNA repression between wild type and *glp-4* mutant worms can be attributed to the germ line. This suggests that in *C. elegans* the germ line is the main compartment of translational repression.

Transcripts that are repressed in *glp-4* mutant animals, i. e. somatically repressed mRNA, can be divided into two subcategories based on their expression in wildtype gonads. mRNAs that are moderately expressed in wildtype animals (wildtype total RNA level < 8) show a similar expression and polysomal association between both worm strains (data not shown). In contrast, somatically repressed mRNAs that are highly expressed in wildtype worms (wildtype total RNA level > 8) are no longer repressed in wildtype animals (Fig. 3). Additionally, these mRNAs are less abundant in germline-less mutant worms compared to wildtype worms, either due to transcriptional repression or mRNA degradation specifically in the soma.

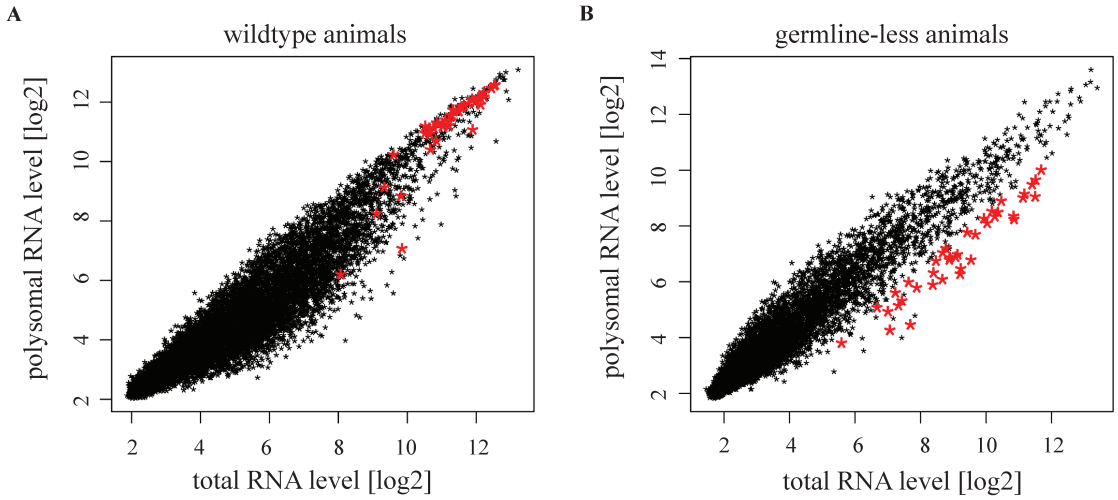

Figure 3
The translational machinery is translated in the germ line but repressed in the soma.
glp-4 repressed mRNAs that are highly expressed in wildtype worms are colored in red (wildtype total RNA level > 8 (log2 scale)). The colored mRNAs encode nearly exclusively for components of the translational machinery and are translated in wildtype animals (A) but repressed in germline-less mutant animals (B).

Looking closer at these 39 mRNAs we found that they encode for 31 ribosomal proteins, the translation elongation factor eEF1γ, the polyA binding protein PAB-1, the homologue of the translational regulator Squid SQD-1 and 5 unknown proteins (Table 1).

gene name	function
rps-1/4/6/7/11/12/18/22/26/30/35/ rpl-5/6/9/13/15/17/18/19/20/21/24.1/ 25.1/30/31/33/34/36 rla-0/1, ubl-1	ribosomal protein
eef-1G	elongation factor eEF1γ
pab-1	polyA binding protein
sqd-1	translational repressor
MTCE.7, MTCE.33 rack-1, Y71F9AL-9	unknown

Table 1
mRNAs that are repressed in germline-less mutants but translated in wildtype worms encode nearly exclusively for components of the translational machinery.

This suggests that while in the germ line the translational machinery is highly expressed and translated, in the soma these mRNAs are downregulated in their abundance and are translationally repressed.

3.1.3. Discussion

It is widely accepted in the field that post-transcriptional gene regulation is widespread in the germ line. Many RBPs and regulated mRNAs have been described and RBPs are generally highly expressed in the germline (Wang et al. 2009). Moreover in contrast to post-transcriptional regulation, transcription has only a minor contribution in recapitulating the expression pattern of several germline proteins (Merritt et al. 2008). The scale of post-transcriptional regulation has yet to be determined.

We found that more than 800 mRNAs are specifically depleted from polysomes in the germ line. Although unlikely, the sub-polysomal association of these transcripts might reflect that these mRNAs are generally poorly translated instead of being targeted by a specific repressive mechanism. We sought to determine the ribosomal association of these mRNAs in germline-less mutant. Most of the mRNAs that are poorly translated in the germ line are however germline-specific. The few mRNAs that are also expressed in the soma are more translated in the soma then in the germline but the small sample size did not allow us to make a general statement about this finding. Other evidence however comes from the global analysis of the ribosomal association in *gld-1* mutants (section 3.2, Fig. 2). Loss of the translational repressor GLD-1 leads to an increased polysomal association of many mRNAs, suggesting that these mRNAs are not generally poorly translated but are repressed via specific mechanisms. We therefore conclude that poorly translated mRNAs represent repressed mRNAs.

Moreover we found that compared to wildtype worms only few mRNAs were associated with sub-polysomal fractions in germline-less worms, indicating that in the adult hermaphrodite, the germ line is the main compartment of translational repression.

Interestingly, many mRNAs that are repressed in the soma encode for components of the translational machinery. Moreover these mRNAs were expressed at a lower level in the soma. One reason for the downregulation of the translation machinery might be that germ cells are the only dividing cells in the adult worm. Moreover, since proteins

that are encoded by maternal mRNAs drive the oocyte-to-embryo transition in the absence of transcription, not only maternal mRNAs but also the translational machinery needs to be inherited to the embryo to ensure that maternal mRNAs can get readily translated when needed.

Concluding, we find that many germline specific mRNAs are repressed in the gonad and while generally only few mRNAs are repressed in the soma, the translational machinery is specifically downregulated in somatic tissues.

3.1.4. Experimental Procedures

Experimental procedures are described in section 3.2.

3.2. GLD-2 binding marks specific mRNA targets for accumulation in oocytes

GLD-1 binding marks specific mRNA targets for accumulation in oocytes

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Running title: **A role of GLD-1 in mRNA storage**

Character count (including spaces): 54371

Summary

Maternal mRNAs loaded into transcriptionally quiescent oocytes are stored in an inactive but stable form, in case of human oocytes for several decades, to support the oocyte-to-embryo transition (OET). These mRNAs are thought to be stable by 'default', due to a global repression of mRNA decay pathways. However, we find that a large group of mRNAs encoding factors driving OET is specifically stabilized in the *C. elegans* germ line by the combined function of two conserved RNA regulators. One of them, the sequence-specific RNA binding protein of the STAR family, GLD-1, represses translation of associated mRNAs. This appears to mark them for stabilization that depends on CGH-1, a DDX6-like RNA helicase, which is a component of germline RNA/protein granules and somatic processing (P) bodies. Our findings suggest that the GLD-1 and CGH-1-dependent pathway for mRNA storage ensures efficient accumulation, and consequently function, of OET regulators.

Highlights

(1) GLD-1 represses translational initiation of its mRNA targets.

(2) GLD-1 has a second role in mRNA stabilization.

(3) The RNA helicase CGH-1 co-regulates the stability of a large group of GLD-1 targets.

(4) GLD-1 and CGH-1 stabilized mRNAs encode regulators of the oocyte-to-embryo transition.

Introduction

The oocyte-to-embryo transition (OET), which encompasses oocyte maturation, ovulation, fertilization, and early embryogenesis, occurs while transcription is globally repressed. For this reason, OET is largely driven by maternal mRNAs, whose translation is controlled both spatially and temporally (reviewed by Lasko, 2009). A long time can separate the production and utilization of maternal mRNAs – up to fifty years in human oocytes. Yet, in contrast to translational repression, which has been extensively studied in several animal models, it is not clear how the stabilization of maternal mRNAs 'stored' in the egg cytoplasm is achieved. *Xenopus* oocytes, for example, lack decapping activity (Zhang et al., 1999), which may explain why deadenylated mRNAs are unusually stable (Voeltz and Steitz, 1998). However, a global repression of mRNA decay may not be a general feature of animal oocytes. For example, in *Drosophila* oocytes, stabilization of the *bicoid* mRNA depends on a specific binding factor, BSF (Mancebo et al., 2001). This suggests that at least some decay pathways are active in oocytes, and that a dedicated mechanism protecting certain messages from degradation must exist.

In *C. elegans*, the DDX6-like RNA helicase, CGH-1, associates with a large number of germline mRNAs (Boag et al., 2008). A few of these mRNAs were shown to be less abundant in the absence of CGH-1, suggesting that these messages are stabilized by CGH-1 (Boag et al., 2008). However, how this helicase, which is thought to have little RNA binding specificity (Linder, 2006), is recruited to specific mRNAs remains unclear. DDX6 helicases can localize to yeast and animal somatic processing (P) bodies (Cougot et al., 2004; Sheth and Parker, 2003), where they activate mRNA decapping and translational repression (Coller and Parker, 2005; Coller et al., 2001). In mouse, frog, fly, and worm germ cells, DDX6 proteins form

mRNA-protein (mRNP) complexes containing Y-box and Sm-like proteins (Boag et al., 2008; Boag et al., 2005; Ladomery et al., 1997; Minshall et al., 2007; Nakamura et al., 2001; Paynton, 1998; Pepling et al., 2007; Wilhelm et al., 2005). In the *C. elegans* germ line, these mRNPs can form microscopically visible P body-like cytoplasmic granules, which were dubbed "storage bodies". In contrast to somatic P bodies, storage bodies seem to be devoid of RNA decay enzymes and are thus thought to serve as vehicles of mRNA storage (Anderson and Kedersha, 2009; Boag et al., 2008; Flemr et al.; Gallo et al., 2008; Lin et al., 2008; Noble et al., 2008; Swetloff et al., 2009). However, because P body formation is thought to be the consequence, not the cause, of mRNA repression, the functional significance of these macroscopic RNA granules remains to be demonstrated (Decker et al., 2007; Eulalio et al., 2007).

Here, we describe a role for the *C. elegans* STAR-domain protein GLD-1 in translation and stabilization of many maternal mRNAs. GLD-1 is expressed in the medial gonad (Fig. 1A), where it promotes meiosis, oogenesis, and maintenance of germ cell identity by repressing translation of diverse mRNAs (Biedermann et al., 2009; Jan et al., 1999; Lee and Schedl, 2001; Marin and Evans, 2003; Mootz et al., 2004; Schumacher et al., 2005). Several mutations that impact on GLD-1 function are within the RNA-binding STAR domain (Francis et al., 1995; Jones and Schedl, 1995), demonstrating that RNA binding is critical for GLD-1 function. We recently found that GLD-1 associates with many germline transcripts, and that this association is determined by 7-mer GLD-1 binding motifs (GBMs) within untranslated regions (UTRs) (Wright et al., 2010). To understand how GLD-1 affects its mRNA targets, we undertook a functional genomics approach. We found that GLD-1 has a widespread function in repressing translational initiation. Unexpectedly, we also uncovered a second role of GLD-1 in mRNA stability. In part, this role depends on

the DDX6 RNA helicase CGH-1, which co-regulates with GLD-1 the stability of many transcripts encoding critical regulators of OET. This study describes a pathway for the stabilization of a functionally related group of maternal mRNAs, demonstrates that stabilization of these mRNAs is important for their accumulation in oocytes, and suggests that in the absence of this pathway OET may be compromised.

Results

GLD-associated mRNAs are mostly not translated

To determine how GLD-1 regulates translation, we analyzed several of its mRNA targets by 'polysomal profiling'. By this approach, poly-ribosomes (polysomes), which are engaged in mRNA translation, are separated from single ribosomes and ribosomal subunits by sucrose density gradient ultracentrifugation (Fig. 1B). In general, efficiently translated mRNAs (as well as mRNAs that are repressed at the level of translational elongation or termination) are found in polysomal (heavy) fractions. Conversely, non-translated mRNAs are present in sub-polysomal (light) fractions. To determine the distribution of GLD-1 between the fractions, we raised monoclonal antibodies against GLD-1. Additionally, we produced monoclonal antibodies against the cytoplasmic polyA-binding protein, PAB-1, which is an activator of mRNA translation. As expected, we found that PAB-1 was enriched in polysomal fractions (Fig. 1C). In contrast, the bulk of GLD-1 was found in sub-polysomal fractions, suggesting that GLD-1 represses translation at the level of initiation and not elongation or termination (Fig. 1C). These distribution patterns correspond to germline proteins, as both GLD-1 and PAB-1 are predominantly expressed in this tissue (Fig. S1A).

The distribution of mRNAs between the fractions was determined by reverse transcription-quantitative PCR (RT-qPCR). Control mRNAs (*act-1*, *elt-2*, *tbb-2*) that do not associate with GLD-1 were largely associated with polysomes and, as expected, this association was lost upon the disruption of polysomes by EDTA-treatment (Figs. 1D, S1B and S1C). To determine the translational status of GLD-1-regulated mRNAs, we chose five well established (*cep-1*, *glp-1*, *pal-1*, *rme-2* and *tra-*

2) and two recently reported (*nos-2* and *pos-1*) GLD-1 targets (Wright et al., 2010; Table S1); these targets are expressed predominantly in the germ line (Fig. S2A), and *nos-2* and *pos-1* also associate with CGH-1 (Boag et al., 2008). In contrast to control mRNAs, we found that only a minor fraction of each GLD-1 mRNA target was present in polysomal fractions (Figs. 1D, S2B), suggesting that these mRNAs are mostly non-translated.

GLD-1 represses translational initiation

To test if the enrichment of GLD-1 and its targets in the sub-polysomal fractions reflects GLD-1-mediated repression of translational initiation, we asked if the distribution of target mRNAs shifts towards polysomal fractions in gradients performed on extracts from *gld-1(q485)* mutant worms (hereafter called *gld-1* mutants). To collect sufficient quantities of mutant animals, *gld-1* homozygous mutants were separated from heterozygous animals carrying a GFP-tagged balancer by fluorescence-activated sorting. We found that the polysomal association of several target mRNAs increased in the *gld-1* mutant (Fig. 2A), suggesting that GLD-1 represses translational initiation of these targets.

To study this role of GLD-1 on a transcriptome-wide level, we measured the polysomal association for all mRNAs in wild-type and *gld-1* mutant worms by microarrays. By comparing polysomal mRNA (fractions 8-12) to total mRNA (fractions 1-12), we calculated a 'polysomal association index', which represents the tendency of an mRNA to be translated. To determine if translation of GLD-1 targets is affected in *gld-1* mutants, we compared the polysomal association indices of GLD-1 target mRNAs (mRNAs that are more than three fold enriched in GLD-1 immunoprecipitates; Fig. S3 and Table S1) and non-target mRNAs. Firstly, we found

that GLD-1 targets were less translated than non-targets in the wild-type animals (Fig. 2B). Secondly, in contrast to non-targets, GLD-1 targets shifted to polysomes in *gld-1* mutants (Fig. 2B; p-value < 7.228e-16). We noticed however that the polysomal association of GLD-1 targets in *gld-1* mutants was still lower than the polysomal association of non-targets. Concluding, GLD-1 appears to repress many targets by preventing translational initiation, but in the *gld-1* mutant translation of its targets continues to be inefficient (see Discussion).

GLD-1 is required for the accumulation of many mRNA targets

Apart from analyzing the relative association of GLD-1 targets with polysomes, we also examined their absolute levels by RT-qPCR. We found that several tested targets, but not control mRNAs, were less abundant in *gld-1* mutants (Fig. 3A). We confirmed this observation by in situ hybridization (Fig. 3B). This finding suggests that in addition to its role in translational repression, GLD-1 has a second role in mRNA stability. To determine how widespread this function of GLD-1 is, we examined changes in the mRNA abundance between wild-type and *gld-1* mutant animals by microarray analysis of total mRNA purified from dissected gonads. A large group of transcripts (410) were more than two-fold less abundant in *gld-1* mutant gonads. Comparing the change in their abundance to GLD-1 binding, we noticed a subtle but significant relation: many mRNAs strongly bound by GLD-1 tend to be less abundant in the *gld-1* mutant (Fig. 3C; transcripts in red), suggesting that the stability of these mRNAs may directly depend on GLD-1 binding.

GLD-1 binding can stabilize its mRNA target

The above experiments were done in *gld-1* mutants that display major germline defects. Also, GLD-1 has been previously suggested to protect mRNAs containing short upstream open reading frames (uORFs) from nonsense-mediated mRNA decay (NMD) by repressing their translation (Lee and Schedl, 2004). Thus, to demonstrate that de-stabilization of at least some GLD-1 targets is caused directly by the loss of GLD-1 binding, rather than by a secondary germline defect, and does not require NMD, we turned to mRNA reporters that are efficiently expressed in the wild-type germ line. Briefly, we used a constitutive germline promoter (*mex-5*) to drive transcription of GFP fused to histone H2B (which concentrates GFP in the nucleus facilitating detection) (Merritt et al., 2008). In designing 3' UTRs, we took advantage of our recent finding that GLD-1 association with mRNAs depends on GLD-1-binding motifs (GBMs), usually found in the 3' UTR (Wright et al., 2010). To determine how GLD-1 binding affects a reporter mRNA, we expressed the reporter under the control of a 3' UTR that either contained wild-type GBMs (GBM*wt*), allowing GLD-1 binding and regulation, or mutated GBMs (GBM*mut*), preventing GLD-1 binding and regulation (Fig. 4A). We tested 3' UTRs belonging to GLD-1 targets that are de-stabilized in *gld-1* mutants, and that encode important regulators of germ line development. Specifically, we chose 3' UTRs from the two most destabilized targets (*gld-1* itself and *egg-1*), from two mRNAs (*rme-2* and *glp-1*) that were used in the above experiments, and additionally from *oma-2*, which is one of the strongest GLD-1 binders (Table S1). To minimize variation between 'GBM*wt*' and 'GBM*mut*' pairs of reporters, transgenic strains were created by Mos1 transposase mediated Single Copy gene Insertion (MosSCI) into a single genomic locus (Frokjaer-Jensen et al., 2008). Strains were examined for GFP-H2B expression patterns in the gonad, and for transcript abundance by RT-qPCR and *in situ* hybridization. In all

cases but one (*gld-1*), mutating GBMs in the 3'UTR caused de-repression of the reporter GFP in the medial (GLD-1-expressing) part of the gonad (Fig. 4B; (Wright et al., 2010; and data not shown). Importantly, in all cases but one (*rme-2*), GBM*mut* transcripts were less abundant by both *in situ* hybridization (Fig. 4C) and RT-qPCR (Fig. 4D). These experiments confirm that GLD-1 binding can induce not only translational repression but also stabilization of some targets.

GLD-1 interacts with conserved components of germline mRNPs

To understand how GLD-1 controls translational repression and mRNA stability, we immunopurified GLD-1 and analyzed co-purifed proteins by mass spectrometry. We found that several conserved RNA regulators were more abundant in GLD-1 immunoprecipitates (IPs) than in control IPs (Figs. 5A and S4). These included the DDX6 RNA helicase CGH-1, the Y-box proteins CEY-1-4, the Sm-related protein CAR-1, and the cytoplasmic polyA binding protein PAB-1. Using available antibodies, we confirmed by western blot analysis the interactions between GLD-1 and CGH-1, CAR-1, and PAB-1 (Fig. 5B). Although these associations were specific, as control proteins did not co-IP with GLD-1 (Fig. 5B), only a small fraction of CGH-1, CAR-1, and PAB-1 interacted with GLD-1, suggesting that these proteins associate with a minor fraction of GLD-1, and/or that the interaction is transient or indirect. To investigate this further, we tested if GLD-1 co-localizes with CGH-1 in the germ cell cytoplasm by immunofluorescence using specific antibodies. CGH-1 staining had a granular appearance as previously reported (Boag et al., 2005). To what extent this pattern reflects the protein distribution in live animals is currently unclear. However, under identical staining conditions only a minor fraction of GLD-1 co-localized with

CGH-1 (Fig. 5C), which is consistent with the weak interaction observed by immunoprecipitation.

CGH-1 promotes stabilization of some GLD-1 targets

To test if the interaction between GLD-1 and CGH-1 reflects a functional relation, we analyzed translation and stability of GLD-1 targets in the temperature-sensitive *cgh-1(tn691)* mutant (hereafter called *cgh-1*). To follow translation, we performed polysome profile analysis on *cgh-1* mutants raised at the restrictive temperature (at this temperature the *tn691* allele phenocopies the null mutation, see Exp.Proc.). Despite fully penetrant oocyte defects, as described for a *cgh-1* null mutant (Boag et al., 2005), the loss of CGH-1 function did not effect translation of most tested GLD-1 targets (Fig. 6A). Consistently with this and previous reports, we found that the proteins GLP-1 and RME-2 (encoded by some of the tested mRNAs) were not de-repressed in the medial gonad (Navarro et al., 2001; data not shown). Additionally, loss of CGH-1 did not appear to affect the level and distribution of GLD-1 (Navarro et al., 2001; Fig. S5A; and data not shown). Conversely, the loss of GLD-1 did not affect CGH-1 (Navarro and Blackwell, 2005; Fig. S5B; and data not shown). Thus, CGH-1 does not appear to play a major role in regulating translation of GLD-1 targets.

Boag et al., 2008 observed that CGH-1 associates with a large set of mRNAs, and showed that six of these mRNAs, including *nos-2* and *pos-1*, are less abundant in the absence of CGH-1. Consistently, we found that mRNA levels of *nos-2* and *pos-1* were reduced in *cgh-1* mutants (Figure 6B). Additionally, we observed that at least two additional GLD-1 targets (*rme-2* and *tra-2*) required CGH-1 for their stability. We confirmed these results by in situ hybridization (Fig. 6C). To examine the extent

of CGH-1-dependent mRNA stabilization, we analyzed mRNAs extracted from dissected gonads by microarrays. The depletion of CGH-1 caused a reproducible change in mRNA abundance, with 748 mRNAs being more than 1.5 fold reduced. This suggests that CGH-1 is required for accumulation of many mRNAs (Table S1).

GLD-1 and CGH-1 stabilize a common set of functionally related mRNAs

To directly compare the effect of GLD-1 and CGH-1 on mRNA stability, we plotted the changes in mRNA levels caused by the loss of GLD-1 against the changes caused by the loss of CGH-1. We found a correlation extending to the lower left quadrant, including 179 mRNAs with lower abundance in both mutants (Fig. 7A; Table S1). Thus, GLD-1 and CGH-1 appear to co-regulate the stability of a large number of mRNAs. Importantly, half of these transcripts (89) are also GLD-1 targets (Fig. 7A; red dots indicate GLD-1 targets), suggesting that destabilization of at least some of these mRNAs is due to the loss of GLD-1 binding.

The DAVID GO term analysis of these 89 mRNAs revealed that they encode proteins implicated in 'embryonic development ending in birth or egg hatching' (Table S2). Some of these proteins (34) have been studied in at least some detail. Interestingly, most of them (30/34) are critical for the oocyte-to-embryo transition (Fig. 7B), with some having specific function during oogenesis (for example PUF-5; Lublin and Evans, 2007), fertilization (EGG-1; Kadandale et al., 2005), or early embryogenesis (POS-1; Tabara et al., 1999). Others, such as OMA-2, function at multiple times during OET (Detwiler et al., 2001; Guven-Ozkan et al., 2008; Shimada et al., 2006). These findings suggest that GLD-1 and CGH-1 co-regulate the stability of specific OET transcripts. Because the loss of GLD-1 appears to affect both translation and stability, while the loss of CGH-1 affects only stability, our model is

that GLD-1-mediated repression 'primes' OET messages for stabilization by CGH-1 (Fig. 7C). Consequently, a message is stored in the cytoplasm in a stable form, which may be critical for its later expression and function, long after GLD-1 has disappeared.

Discussion

GLD-1-mediated repression of translational initiation

Several mRNAs were shown to be translationally repressed by GLD-1. One of them, *tra-2*, was suggested to be repressed at the initiation stage of translation (Goodwin et al., 1993), while another, *pal-1*, at the elongation stage (Mootz et al., 2004). Although it remains possible that GLD-1 represses translation by different mechanisms, our global analysis is consistent with GLD-1 repressing mostly translational initiation. Interestingly, in the *gld-1* mutant, GLD-1 targets continue to be less efficiently translated than non-associated mRNAs. Although the reason for this is currently unknown, one possibility is that these messages are co-regulated by repressors that function redundantly with GLD-1. Alternatively or additionally, proteins such as the KH-domain protein MEX-3, which in the distal-most gonad inhibits specific GLD-1 targets and in the medial gonad is repressed by GLD-1 (Ciosk et al., 2004; Mootz et al., 2004), may repress GLD-1 targets when de-repressed in the *gld-1* mutant. The precise mechanism of GLD-1-mediated repression remains unknown. We found that GLD-1 interacts with the DDX6 helicase (CGH-1) and the Y-box proteins (CEY-1 – 4). In frog and fly oocytes, these proteins are part of a large mRNP complex repressing translation in a 5' cap-dependent manner, by recruiting a 4E binding protein (4E-BP) that prevents assembly of the basic translational initiation factor eIF4F (Kim-Ha et al., 1995; Ladomery et al., 1997; Minshall and Standart, 2004; Nakamura et al., 2001; Nakamura et al., 2004; Stebbins-Boaz et al., 1999). In this complex, mRNA-binding specificity appears to come from the associated sequence-specific RBPs, the cytoplasmic polyadenylation element binding protein CPEB and the RRM-domain protein Bruno. In contrast, GLD-1, which may similarly provide mRNA specificity for CGH-1 and CEY proteins, does not appear to interact with the

C. elegans 4E-BP, SPN-2 (Li et al., 2009), or with translation initiation factors. Interestingly, Me31B and Bruno repress *oskar* mRNA also in a 5'cap-independent manner by sequestering it away from the translational machinery, which may involve mRNA oligomerization (Chekulaeva et al., 2006). It is possible that a similar mechanism, where the translational apparatus cannot access sequestered mRNA, may be used in the worm germ line. However, what complicates this model is the observation that, in contrast to the frog and fly DDX6 proteins (Minshall and Standart, 2004; Nakamura et al., 2001), CGH-1 alone does not appear to repress translation of several tested mRNAs (this study; Navarro et al., 2001).

The relation between GLD-1 and CGH-1

Despite the functional cooperation between GLD-1 and CGH-1, the corresponding mutants have largely distinct phenotypes. While *gld-1* adult gonads, among other defects, develop tumors containing both proliferating germ cells and ectopic somatic (teratomatous) cells (Ciosk et al., 2006; Francis et al., 1995), *cgh-1* gonads are mostly defective in oocyte formation (Navarro et al., 2001). These differences may, on one hand, reflect de-regulation of messages controlled specifically by either GLD-1 or CGH-1, possibly in conjunction with additional partners. On the other hand, the phenotypic differences may reflect mechanistic differences between GLD-1 and CGH-1. GLD-1 appears to regulate both translation and stabilization, so de-regulation of individual targets will contribute to the phenotype both through ectopic translation (a gain-of function-like phenotype), and through transcript destabilization by currently unknown decay mechanism(s) (a loss of function-like phenotype). In contrast, the *cgh-1* phenotype may be largely due to transcript destabilization and, consequently, decreased protein levels.

GLD-1 and CGH-1-mediated storage of OET mRNAs

Our model is that GLD-1-mediated repression precedes CGH-1-dependent mRNA stabilization, possibly through mRNA sequestration into storage mRNPs, where mRNAs might no longer be accessible to the mRNA decay machinery. Interestingly, the protist DDX6-like helicase, DOZI, has been shown to stabilize repressed transcripts in female gametocytes (Mair et al., 2006). Thus, the role of DDX6 helicases in mRNA stability may be a conserved feature of germ cells.

GLD-1 and CGH-1-stabilized mRNAs encode many critical regulators of OET. In the absence of GLD-1/CGH-1, these mRNAs are prematurely translated but importantly also degraded. Thus, GLD-1 and CGH-1 mediated mRNA storage is expected to ensure error-free OET by preventing both precocious translation and insufficient accumulation of OET regulators. Whether the pathway for the storage of OET transcripts described here operates in other animals is currently unknown. Intriguingly however, expression profiling experiments on mouse oocytes revealed a correlation between aging and a decrease in some maternal effect transcripts (Hamatani et al., 2004), which can be considered the functional equivalents of worm OET mRNAs. Thus, it is tempting to speculate that a pathway analogous to the one described here may contribute to the quality of human oocytes by ensuring correct storage (repression but also stabilization) of specific maternal transcripts.

Experimental Procedures

Nematode culture, mutants, transgenic strains, and worm sorting

Animals were maintained at 25°C using standard procedures, unless indicated otherwise. The temperature sensitive strains *glp-4(bn2)* and *cgh-1(tn691)* were maintained at 15°C. Synchronous cultures were obtained by collecting eggs from bleached adults and synchronizing larvae by starvation before feeding. Young adults that produced oocytes but not embryos were used in most experiments.

The following mutant and transgenic strains have been described previously: *gld-1(q485)/hT2[qIs48]*; *glp-4(bn2); rrrSi 37- 40 [mex-5 pro::PEST:GFP-H2B::oma-2 3'UTR; unc-119(+)]II; and rrrSi 53 -57[mex-5 pro::PEST:GFP-H2B::oma-2 GBM mut 3'UTR; unc-119(+)]II* (Beanan and Strome, 1992; Ciosk et al., 2006; Wright et al., 2010). *cgh-1(tn691)* was obtained from CGC (DG1701) and displays at the restrictive temperature (25°C) a similar phenotype as *cgh-1(ok492)/hT2[qIs48]* (Boag et al., 2005).

Single copy-integrated constructs were generated as previously described (Frokjaer-Jensen et al., 2008; Wright et al., 2010), with the following modification: constructs containing *glp-1* and *rme-2* 3' UTRs were fused to GFP-H2B (from pCM1.35). Oligos used to amplify 3' UTR sequences (from the STOP codon to 50bp downstream of the polyA site) are described in Table S3. All strains were outcrossed at least twice against wild-type worms before being analyzed (Table S4).

We used the COPAS^{TM} Biosort from Union Biometrica to separate homozygous GFP (-) *gld-1* mutants from heterozygous GFP (+) *gld-1(q485)/hT2[qIs48]* animals.

Polysome profile analysis and isolation of RNA and proteins

The assay was performed as previously described (Ding and Grosshans, 2009), with the following changes. Synchronized worms were harvested as young adults, frozen in 100 µl 'worm pellet' aliquots. Subsequently, each aliquot was re-suspended in 500 µl lysis buffer. An initial centrifugation step was included (5min at 5000g, 4°C) and worm lysates were layered on 5% (w/v) to 45% (w/v) sucrose gradients. To correct for variations in RNA isolation and reverse transcription efficiency between sucrose fractions, we added 2 µg of total RNA from mouse brain (Stratagene) to each fraction. RNA from fractions was extracted using TRIzol (Invitrogen) according to the manufacturer's recommendations. RNA integrity was confirmed on ethidium bromide-stained agarose gels before proceeding to RT. To examine total mRNA levels in wild-type, *cgh-1(tn691)*, *gld-1(q485)*, and *glp-4(bn2)* mutant animals, RNA was extracted from lysates used for polysome profile. Proteins from fractions were isolated by chloroform/methanol precipitation and investigated by western blotting. To analyze mRNAs by tiling arrays, we extracted RNA from pooled fractions 8 to 12 (polysomal) and fractions 1 – 12 (total), in four biological replicates.

RNA isolation from whole animals or dissected gonads

Synchronized young adults were washed twice in cold M9 and frozen as 50 µl pellets. Worm pellets were resuspended in 900 µl TRIzol, and crushed to a fine powder with a mortar and a pestle. RNA was extracted according to the manufacturer's recommendations.

50 gonads from wild-type, *gld-1(q485)*, or *cgh-1 (tn691)* worms were dissected in triplicates in M9 buffer. RNA was isolated using the PicoPure™ RNA Isolation Kit (Applied Biosystems), according to the manufacturer's recommendations.

Antibodies

Peptides (Bachem) were used to generate mouse monoclonal antibodies according to standard procedures (PAB-1 = aa 542 – 560; GLD-1 = aa 65 - 79). Antibodies were diluted 1:50 for western blot analysis, GLD-1 antibody was diluted 1:500 for immunostainings. Additional antibodies used: ACT-1 (MAB1501, Chemicon), CAR-1, CGH-1 (Boag et al., 2005), FLAG M2 (Sigma), GLH-1 (Orsborn et al., 2007), Myc (9E10), PGL-1 (Kawasaki et al., 1998).

GLD-1 immunoprecipitation and analysis of co-precipitated RNA

GLD-1 immunoprecipitations were performed as previously described (Biedermann et al., 2009). GLD-1 targets were identified by comparing anti-GLD-1 IPs with anti-Myc IPs. RNA was eluted from beads with TRIzol. Precipitation efficiency was enhanced by adding 5 μg total RNA from mouse brain (Stratagene) to each IP sample.

GLD-1 immunoprecipitation and analysis of co-precipitated proteins

GLD-1-associated proteins were identified by comparing anti-GLD-1 IPs with anti-FLAG IPs. Proteins were separated by SDS-PAGE and Coomassie stained. Bands were cut, washed and in-gel digested with trypsin overnight at 37 °C. Tryptic peptides were separated on an Agilent 1100 nanoLC system (Agilent Technologies) coupled to an LTQ Orbitrap Velos hybrid mass spectrometer (Thermo Scientific). The LC system was equipped with a Peptide CapTrap column (Michrom BioResources, Inc.) and a capillary column with integrated nanospray tip (75 mm i.d. x 100 mm, Swiss BioAnalytics AG) filled with MagicC18 (Michrom Bioresources, Inc.). Elution was performed with a gradient of 0 - 45% solvent B in 30 min at a flow rate of 400 nl/

min. Solvent A consisted of 0.1% formic acid/ 2% acetonitrile, solvent B was composed of 0.1% formic acid/ 80% acetonitrile. The mass spectrometer operated in positive mode using the top 20 DDA method. Peptides were identified searching UniProt 15.14 using Mascot Distiller 2.3 and Mascot 2.2 (Matrix Science). Results were compiled in Scaffold 2.06. (Proteome Software).

RT-qPCR

Reverse transcription reactions were performed using the ImProm-II™ Reverse Transcription System (Promega). To ensure that we are detecting full-length, polyadenylated transcripts we used oligo $dT_{(15)}$ primers for RT reactions on RNA from polysome profile fractions. Identical results were obtained using random hexamer oligonucleotides. To compare total mRNA levels and analyze GLD-1 co-immunoprecipitated RNA, cDNA was generated using random hexamer primers. qPCR reactions were performed as described previously (Biedermann et al., 2009). At least one primer in each pair is specific for an exon-exon junction (Table S3). Mouse RNA (*Cyt-c*) was added before RNA isolation and RT, allowing us to normalize all obtained qPCR results to *Cyt-c*, thereby correcting for variations in RNA isolation and RT. To compare total mRNA levels, qPCR results were normalized to *act-1* and wild-type values. GLD-1 IP enrichment was calculated relative to the control IP.

RNA hybridization to tiling arrays

300 ng of RNA (IP and pooled gradient fractions) or 5 µl of RNA (corresponding to 25 dissected gonads and isolated with the PicoPure Kit) were amplified once into dsDNA. Then, 7.5µg of cDNA was subsequently fragmented and labeled according to the "GeneChip Expression Analysis Technical Manual" (Affymetrix). 6 µg of

fragmented and labeled DNA were hybridized to the Affymetrix *C. elegans* tiling array chip according to the Affymetrix Expression Analysis Technical Manual. Microarray sample preparation, hybridization and scanning were performed in the FMI genomics facility.

Analysis of tiling array data

Tiling arrays were processed in R (www.r-project.org; (Ihaka and Gentleman, 1996) using bioconductor (Gentleman et al., 2004), and the packages tilingArray (Huber et al., 2006) and preprocessCore. The arrays were RMA background corrected and log2 transformed on the oligo level using the command:

```
expr <-log2
(rma.background.correct(exprs(readCel2eSet(filenames,rotated=TRUE)))).
```

We mapped the oligos from the tiling array (bpmap file from www.affymetrix.com) to the *C. elegans* genome assembly ce6 (www.genome.ucsc.edu) using bowtie (Langmead et al., 2009) allowing no error and unique mapping position. Expression of individual transcripts was calculated by intersecting the genomic positions of oligos with transcript annotation (WormBase WS190) and averaging the intensity of the respective oligos. Quantile normalization: each of the 3 datasets was processed with an individual quantile normalization scheme. For IP experiments, no quantile normalization was performed as the distributions between GLD-1 IPs and control IPs differs substantially. In the case of the polysome dataset (containing polysome and total RNA samples) quantile normalization was performed twice. Once containing all the polysome samples and once for all the total RNA samples. The third dataset containing only total RNA from purified gonads was quantile normalized in one single step.

RNA in situ hybridization, immunolocalization, and microscopy

RNA in situ hybridization was performed and analyzed as previously described (Biedermann et al., 2009). The probes generated from cDNA correspond to nt 816 – 1369 (*glp-1*); 150 – 699 (*nos-2*); 118 - 611 (*pal-1*); 85 – 611 (*pos-1*); 106 – 635 (*rme-2);* and 1 – 714 (gfp) (Table S3). Unless indicated otherwise, images were captured with a Zeiss AxioImager Z1 microscope, equipped with an Axiocam MRm REV2 CCD camera. Images were acquired in the linear mode of the Axiovision software (Zeiss) and processed with Adobe Photoshop CS4 in an identical manner.

Confocal microscopy and deconvolution

A LSM700 confocal microscope equipped with a Plan-Apochromat 63x/1.40 Oil DIC M27 objective was used to capture images with a voxel size of 0.052 μm x 0.052 μm x 0.2 μm (x, y, z). Used lasers: track 1: 405 nm (2%) and 555 nm (10%); track 2: 488 nm (4%). Beam splitters: MBS 405/488/555/639; DBS1: 531 nm (track1) and 578 nm (track 2). Filters: SP490 (Track 1,Channel 1); LP 560 (Track 1, Channel 2); 0 – 587 (Track 2, Channel 1). Pinhole: 40 μm (Track 1); 41 μm (Track 2). Pictures were deconvolved with the Huygens software, using Remote Manager v1.2.3, a SNR of 8, 8, 8, 100 iterations, and the cmle deconvolution algorithm (quality change stopping criterion: 0.1). Deconvolved images were processed in Imaris XP 7.1.1 using the coloc function.

References

Anderson, P., and Kedersha, N. (2009). RNA granules: post-transcriptional and epigenetic modulators of gene expression. Nat Rev Mol Cell Biol *10*, 430-436.

Beanan, M.J., and Strome, S. (1992). Characterization of a germ-line proliferation mutation in C. elegans. Development *116*, 755-766.

Biedermann, B., Wright, J., Senften, M., Kalchhauser, I., Sarathy, G., Lee, M.H., and Ciosk, R. (2009). Translational repression of cyclin E prevents precocious mitosis and embryonic gene activation during C. elegans meiosis. Dev Cell *17*, 355-364.

Boag, P.R., Atalay, A., Robida, S., Reinke, V., and Blackwell, T.K. (2008). Protection of specific maternal messenger RNAs by the P body protein CGH-1 (Dhh1/RCK) during Caenorhabditis elegans oogenesis. J Cell Biol *182*, 543-557.

Boag, P.R., Nakamura, A., and Blackwell, T.K. (2005). A conserved RNA-protein complex component involved in physiological germline apoptosis regulation in C. elegans. Development *132*, 4975-4986.

Chekulaeva, M., Hentze, M.W., and Ephrussi, A. (2006). Bruno acts as a dual repressor of oskar translation, promoting mRNA oligomerization and formation of silencing particles. Cell *124*, 521-533.

Ciosk, R., DePalma, M., and Priess, J.R. (2004). ATX-2, the C. elegans ortholog of ataxin 2, functions in translational regulation in the germline. Development *131*, 4831-4841.

Ciosk, R., DePalma, M., and Priess, J.R. (2006). Translational regulators maintain totipotency in the Caenorhabditis elegans germline. Science *311*, 851-853.

Coller, J., and Parker, R. (2005). General translational repression by activators of mRNA decapping. Cell *122*, 875-886.

Coller, J.M., Tucker, M., Sheth, U., Valencia-Sanchez, M.A., and Parker, R. (2001). The DEAD box helicase, Dhh1p, functions in mRNA decapping and interacts with both the decapping and deadenylase complexes. RNA *7*, 1717-1727.

Cougot, N., Babajko, S., and Seraphin, B. (2004). Cytoplasmic foci are sites of mRNA decay in human cells. J Cell Biol *165*, 31-40.

Decker, C.J., Teixeira, D., and Parker, R. (2007). Edc3p and a glutamine/asparagine-rich domain of Lsm4p function in processing body assembly in Saccharomyces cerevisiae. J Cell Biol *179*, 437-449.

Detwiler, M.R., Reuben, M., Li, X., Rogers, E., and Lin, R. (2001). Two zinc finger proteins, OMA-1 and OMA-2, are redundantly required for oocyte maturation in C. elegans. Dev Cell *1*, 187-199.

Ding, X.C., and Grosshans, H. (2009). Repression of C. elegans microRNA targets at the initiation level of translation requires GW182 proteins. EMBO J *28*, 213-222.

Eulalio, A., Behm-Ansmant, I., Schweizer, D., and Izaurralde, E. (2007). P-body formation is a consequence, not the cause, of RNA-mediated gene silencing. Mol Cell Biol *27*, 3970-3981.

Flemr, M., Ma, J., Schultz, R.M., and Svoboda, P. P-body loss is concomitant with formation of a messenger RNA storage domain in mouse oocytes. Biol Reprod *82*, 1008-1017.

Francis, R., Barton, M.K., Kimble, J., and Schedl, T. (1995). gld-1, a tumor suppressor gene required for oocyte development in Caenorhabditis elegans. Genetics *139*, 579-606.

Frokjaer-Jensen, C., Davis, M.W., Hopkins, C.E., Newman, B.J., Thummel, J.M., Olesen, S.P., Grunnet, M., and Jorgensen, E.M. (2008). Single-copy insertion of transgenes in Caenorhabditis elegans. Nat Genet *40*, 1375-1383.

Gallo, C.M., Munro, E., Rasoloson, D., Merritt, C., and Seydoux, G. (2008). Processing bodies and germ granules are distinct RNA granules that interact in C. elegans embryos. Dev Biol *323*, 76-87.

Gentleman, R.C., Carey, V.J., Bates, D.M., Bolstad, B., Dettling, M., Dudoit, S., Ellis, B., Gautier, L., Ge, Y., Gentry, J., *et al*. (2004). Bioconductor: open software development for computational biology and bioinformatics. Genome Biol *5*, R80.

Goodwin, E.B., Okkema, P.G., Evans, T.C., and Kimble, J. (1993). Translational regulation of tra-2 by its 3' untranslated region controls sexual identity in C. elegans. Cell *75*, 329-339.

Guven-Ozkan, T., Nishi, Y., Robertson, S.M., and Lin, R. (2008). Global transcriptional repression in C. elegans germline precursors by regulated sequestration of TAF-4. Cell *135*, 149-160.

Hamatani, T., Falco, G., Carter, M.G., Akutsu, H., Stagg, C.A., Sharov, A.A., Dudekula, D.B., VanBuren, V., and Ko, M.S. (2004). Age-associated alteration of gene expression patterns in mouse oocytes. Hum Mol Genet *13*, 2263-2278.

Huber, W., Toedling, J., and Steinmetz, L.M. (2006). Transcript mapping with high-density oligonucleotide tiling arrays. Bioinformatics *22*, 1963-1970.

Ihaka, R., and Gentleman, R. (1996). A language for Data Analysis and Graphics . Journal of Computational and Graphical Statistics *5*, 299-314.

Jan, E., Motzny, C.K., Graves, L.E., and Goodwin, E.B. (1999). The STAR protein, GLD-1, is a translational regulator of sexual identity in Caenorhabditis elegans. EMBO J *18*, 258-269.

Jones, A.R., and Schedl, T. (1995). Mutations in gld-1, a female germ cell-specific tumor suppressor gene in Caenorhabditis elegans, affect a conserved domain also found in Src-associated protein Sam68. Genes & development *9*, 1491-1504.

Kadandale, P., Stewart-Michaelis, A., Gordon, S., Rubin, J., Klancer, R., Schweinsberg, P., Grant, B.D., and Singson, A. (2005). The egg surface LDL receptor repeat-containing proteins EGG-1 and EGG-2 are required for fertilization in Caenorhabditis elegans. Curr Biol *15*, 2222-2229.

Kawasaki, I., Shim, Y.H., Kirchner, J., Kaminker, J., Wood, W.B., and Strome, S. (1998). PGL-1, a predicted RNA-binding component of germ granules, is essential for fertility in C. elegans. Cell *94*, 635-645.

Kim-Ha, J., Kerr, K., and Macdonald, P.M. (1995). Translational regulation of oskar mRNA by bruno, an ovarian RNA-binding protein, is essential. Cell *81*, 403-412.

Ladomery, M., Wade, E., and Sommerville, J. (1997). Xp54, the Xenopus homologue of human RNA helicase p54, is an integral component of stored mRNP particles in oocytes. Nucleic Acids Res *25*, 965-973.

Langmead, B., Trapnell, C., Pop, M., and Salzberg, S.L. (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol *10*, R25.

Lasko, P. (2009). Chapter 6 translational control during early development. Prog Mol Biol Transl Sci *90*, 211-254.

Lee, M.H., and Schedl, T. (2001). Identification of in vivo mRNA targets of GLD-1, a maxi-KH motif containing protein required for C. elegans germ cell development. Genes Dev *15*, 2408-2420.

Lee, M.H., and Schedl, T. (2004). Translation repression by GLD-1 protects its mRNA targets from nonsense-mediated mRNA decay in C. elegans. Genes Dev *18*, 1047-1059.

Li, W., DeBella, L.R., Guven-Ozkan, T., Lin, R., and Rose, L.S. (2009). An eIF4E-binding protein regulates katanin protein levels in C. elegans embryos. J Cell Biol *187*, 33-42.

Lin, M.D., Jiao, X., Grima, D., Newbury, S.F., Kiledjian, M., and Chou, T.B. (2008). Drosophila processing bodies in oogenesis. Dev Biol *322*, 276-288.

Linder, P. (2006). Dead-box proteins: a family affair--active and passive players in RNP-remodeling. Nucleic Acids Res *34*, 4168-4180.

Lublin, A.L., and Evans, T.C. (2007). The RNA-binding proteins PUF-5, PUF-6, and PUF-7 reveal multiple systems for maternal mRNA regulation during C. elegans oogenesis. Dev Biol *303*, 635-649.

Mair, G.R., Braks, J.A., Garver, L.S., Wiegant, J.C., Hall, N., Dirks, R.W., Khan, S.M., Dimopoulos, G., Janse, C.J., and Waters, A.P. (2006). Regulation of sexual development of Plasmodium by translational repression. Science *313*, 667-669.

Mancebo, R., Zhou, X., Shillinglaw, W., Henzel, W., and Macdonald, P.M. (2001). BSF binds specifically to the bicoid mRNA 3' untranslated region and contributes to stabilization of bicoid mRNA. Mol Cell Biol *21*, 3462-3471.

Marin, V.A., and Evans, T.C. (2003). Translational repression of a C. elegans Notch mRNA by the STAR/KH domain protein GLD-1. Development *130*, 2623-2632.

Merritt, C., Rasoloson, D., Ko, D., and Seydoux, G. (2008). 3' UTRs are the primary regulators of gene expression in the C. elegans germline. Curr Biol *18*, 1476-1482.

Minshall, N., Reiter, M.H., Weil, D., and Standart, N. (2007). CPEB interacts with an ovary-specific eIF4E and 4E-T in early Xenopus oocytes. J Biol Chem *282*, 37389-37401.

Minshall, N., and Standart, N. (2004). The active form of Xp54 RNA helicase in translational repression is an RNA-mediated oligomer. Nucleic Acids Res *32*, 1325-1334.

Mootz, D., Ho, D.M., and Hunter, C.P. (2004). The STAR/Maxi-KH domain protein GLD-1 mediates a developmental switch in the translational control of C. elegans PAL-1. Development *131*, 3263-3272.

Nakamura, A., Amikura, R., Hanyu, K., and Kobayashi, S. (2001). Me31B silences translation of oocyte-localizing RNAs through the formation of cytoplasmic RNP complex during Drosophila oogenesis. Development *128*, 3233-3242.

Nakamura, A., Sato, K., and Hanyu-Nakamura, K. (2004). Drosophila cup is an eIF4E binding protein that associates with Bruno and regulates oskar mRNA translation in oogenesis. Dev Cell *6*, 69-78.

Navarro, R.E., and Blackwell, T.K. (2005). Requirement for P granules and meiosis for accumulation of the germline RNA helicase CGH-1. Genesis *42*, 172-180.

Navarro, R.E., Shim, E.Y., Kohara, Y., Singson, A., and Blackwell, T.K. (2001). cgh-1, a conserved predicted RNA helicase required for gametogenesis and protection from physiological germline apoptosis in C. elegans. Development *128*, 3221-3232.

Noble, S.L., Allen, B.L., Goh, L.K., Nordick, K., and Evans, T.C. (2008). Maternal mRNAs are regulated by diverse P body-related mRNP granules during early Caenorhabditis elegans development. J Cell Biol *182*, 559-572.

Orsborn, A.M., Li, W., McEwen, T.J., Mizuno, T., Kuzmin, E., Matsumoto, K., and Bennett, K.L. (2007). GLH-1, the C. elegans P granule protein, is controlled by the JNK KGB-1 and by the COP9 subunit CSN-5. Development *134*, 3383-3392.

Paynton, B.V. (1998). RNA-binding proteins in mouse oocytes and embryos: expression of genes encoding Y box, DEAD box RNA helicase, and polyA binding proteins. Dev Genet *23*, 285-298.

Pepling, M.E., Wilhelm, J.E., O'Hara, A.L., Gephardt, G.W., and Spradling, A.C. (2007). Mouse oocytes within germ cell cysts and primordial follicles contain a Balbiani body. Proc Natl Acad Sci U S A *104*, 187-192.

Schumacher, B., Hanazawa, M., Lee, M.H., Nayak, S., Volkmann, K., Hofmann, E.R., Hengartner, M., Schedl, T., and Gartner, A. (2005). Translational repression of C. elegans p53 by GLD-1 regulates DNA damage-induced apoptosis. Cell *120*, 357-368.

Sheth, U., and Parker, R. (2003). Decapping and decay of messenger RNA occur in cytoplasmic processing bodies. Science *300*, 805-808.

Shimada, M., Yokosawa, H., and Kawahara, H. (2006). OMA-1 is a P granules-associated protein that is required for germline specification in Caenorhabditis elegans embryos. Genes Cells *11*, 383-396.

Stebbins-Boaz, B., Cao, Q., de Moor, C.H., Mendez, R., and Richter, J.D. (1999). Maskin is a CPEB-associated factor that transiently interacts with elF-4E. Mol Cell *4*, 1017-1027.

Swetloff, A., Conne, B., Huarte, J., Pitetti, J.L., Nef, S., and Vassalli, J.D. (2009). Dcp1-bodies in mouse oocytes. Mol Biol Cell *20*, 4951-4961.

Tabara, H., Hill, R.J., Mello, C.C., Priess, J.R., and Kohara, Y. (1999). pos-1 encodes a cytoplasmic zinc-finger protein essential for germline specification in C. elegans. Development *126*, 1-11.

Voeltz, G.K., and Steitz, J.A. (1998). AUUUA sequences direct mRNA deadenylation uncoupled from decay during Xenopus early development. Mol Cell Biol *18*, 7537-7545.

Wilhelm, J.E., Buszczak, M., and Sayles, S. (2005). Efficient protein trafficking requires trailer hitch, a component of a ribonucleoprotein complex localized to the ER in Drosophila. Dev Cell *9*, 675-685.

Wright, J.E., Gaidatzis, D., Senften, M., Farley, B.M., Westhof, E., Ryder, S.P., and Ciosk, R. (2010). A quantitative RNA code for mRNA target selection by the germline fate determinant GLD-1. The EMBO Journal advance online publication, 17 Dec 2010. DOI 10.1038/emboj.2010.334

Zhang, S., Williams, C.J., Wormington, M., Stevens, A., and Peltz, S.W. (1999). Monitoring mRNA decapping activity. Methods *17*, 46-51.

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Figure Legends

Figure 1

GLD-1 target mRNAs are largely not translated. (A) Schematic of a *C. elegans* gonad and the oocyte-to-embryo transition (OET). Germline stem cells are found in the distal-most gonad, which is marked here and in subsequent figures by an asterisk. The medial gonad contains germ cells undergoing meiosis and expressing GLD-1. Growing oocytes are present in the proximal gonad. OET, including ovulation, fertilization, and early stages of embryonic development, occurs while transcription is globally repressed. (B) A typical polysome profile derived from young adults. Ultracentrifuged worm extracts were fractionated into 12 fractions. Arrows indicate the positions of monosomes (fraction 7) and polysomes (fractions 8 – 12). The bottom picture shows ribosomal RNAs isolated from each fraction, resolved on an agarose gel. (C) GLD-1 is largely not associated with polysomal mRNAs. Western blot analysis of GLD-1 and PAB-1 on proteins isolated from individual fractions. Compared to the translational activator PAB-1, GLD-1 is enriched in sub-polysomal fractions. (D) Several GLD-1 targets are mostly non-translated. Abundance of indicated mRNAs in the polysomal fractions (8 – 12), relative to the total mRNA (fractions 1 – 12) was measured by reverse-transcription and quantitative PCR (RT-qPCR). mRNA levels were normalized to mouse RNA, which was added to each fraction. Error bars, here and in subsequent figures, represent SEM of at least three biological replicates. See also Figures S1 and S2.

Figure 2

GLD-1 has a global role in repressing translational initiation. (A) The translation of several GLD-1-associated mRNAs increases in *gld-1* mutants. Bars represent relative

changes in the polysomal association of indicated mRNAs in *gld-1(q485)* mutants, relative to wild-type animals. (B) GLD-1 target mRNAs are more likely translated in *gld-1* mutants. Polysomal association indices were determined by comparing polysomal mRNA (fractions 8-12) to total mRNA (fractions 1-12), both of which were examined by microarrays. GLD-1 mRNA targets were determined by microarray analysis of mRNAs that co-purified with the endogenous GLD-1 (Fig. S3A; mRNAs enriched > 3 fold in the IP). Box plots represent the distribution of polysomal association indices for GLD-1 targets and non-targets in wild-type and *gld-1* mutant worms. Association of non-targets with polysomes is similar in wild-type and *gld-1* mutant worms (left panel; p-value = 0.6173). In contrast, GLD-1 targets shift to polysomes in *gld-1* mutants (right panel; p-value = 7.228e-16). See also Figure S3 and Table S1.

Figure 3

GLD-1 is required for the accumulation of its mRNA targets. (A) Several GLD-1 targets are less abundant in *gld-1* mutants. Abundance of indicated mRNAs was determined by RT-qPCR and normalized to a somatic mRNA (*elt-2*). mRNA levels in *gld-1* mutants are shown relative to the levels in wild-type animals. (B) In situ hybridization against indicated GLD-1 targets, performed on gonads dissected from wild type and *gld-1* mutants. Sense controls against all mRNAs were negative (data not shown). (C) Many additional GLD-1 targets are less abundant in *gld-1* mutants. mRNA abundance in dissected wild-type and *gld-1* mutant gonads was measured by microarrays. The change in mRNA abundance in *gld-1* mutant versus wild type was plotted against mRNA enrichment in GLD-1 IPs. Each dot represents a single

transcript. GLD-1 target mRNAs (IP enrichment > three fold), whose abundance decreases in the absence of GLD-1 more than two fold, are marked in red.

Figure 4

GLD-1 binding is required for translational repression and mRNA stabilization. (A) Schematic of reporters that were used to test the effect of GLD-1 binding motifs (GBMs) on mRNA translation and stabilization. P: a germ line-specific promoter (*mex-5*); R-CDS: reporter's coding sequence consisting of a GFP fused to the histone H2B; 3'UTR: contained either a wild-type GBM (GBM*wt*), or a mutated GBM (GBM*mut*) that fails to bind GLD-1. (B) Mutating GBMs causes reporter de-repression in the medial gonad. Shown are photomicrographs of gonads (outlined; red highlighting repressed regions) from live, transgenic, and otherwise wild-type worms. The *egg-1* GBM*wt* reporter is repressed by GLD-1 in the medial gonad, while the corresponding GBM*mut* reporter is de-repressed in the same region. Several other reporter pairs show analogous expression patterns (Wright et al., 2010). (C) Mutating GBMs reduces reporter mRNA levels. The levels of several GBM*wt* and GBM*mut* reporter pairs were determined by in situ hybridization against *gfp* RNA. As a control we used wild type worms, not expressing a GFP reporter (data not shown). In all cases but one (*rme-2*), mutating GBMs destabilized the corresponding reporter. (D) RT-qPCR confirmed that levels of GBM*mut* reporters were reduced relative to GBM*wt* reporters. mRNA levels were normalized to *act-1* mRNA.

Figure 5

GLD-1 interacts with several conserved RNA regulators. (A) A summary of nine GLD-1 interactors identified by mass spectrometry analysis of GLD-1 co-precipitated

proteins, and their homologs from several species. '?' indicates no described homolog. (B) Confirmation of some interactions by western blot analysis of GLD-1 IPs. GLH-1/Vasa and ACT-1/actin are negative controls. (C) GLD-1 and CGH-1 are largely present in distinct cytoplasmic foci. Confocal microscopy on dissected wild-type gonads that were immunostained for GLD-1 and CGH-1. Pictures were deconvolved and shown is a fragment from the medial germ line, whose approximate location is marked by the red square on the schematic gonad. See also Figure S4.

Figure 6

CGH-1 regulates mRNA stability but not translational repression of some GLD-1 targets. (A) Bars represent relative changes in the polysomal association of indicated mRNAs in *cgh-1(tn691)* mutant worms, relative to wild type worms. The polysomal association of most GLD-1 targets is similar between both strains, indicating that GLD-1 can repress translation initiation independently of CGH-1. (B) Several GLD-1 targets are less abundant in *cgh-1* mutants. Abundance of indicated mRNAs in wild-type and *cgh-1* mutant animals was determined by RT-qPCR and normalized to a somatic mRNA, *elt-2*. The levels in *cgh-1* mutants are shown relative to wild-type levels. (C) In situ hybridization against indicated GLD-1 targets, performed on gonads dissected from wild type and *cgh-1* mutants. Sense controls against all mRNAs were negative (data not shown). See also Figure S5.

Figure 7

GLD-1 and CGH-1 co-regulate mRNAs that are required for the oocyte-to-embryo transition. (A) GLD-1 and CGH-1 stabilize common mRNAs. mRNA levels in dissected wild-type, *gld-1*, and *cgh-1* mutant gonads were measured by microarrays.

Change in mRNA abundance in *gld-1* mutant versus wild type was plotted against the change in mRNA abundance in *cgh-1* mutants versus wild type. Each dot represents a single transcript. GLD-1 targets are marked in red. A large number of mRNAs is destabilized in both mutants (present in the lower left quadrant of the plot), many of which are GLD-1 targets. (B) GLD-1 and CGH-1 co-regulated mRNAs function during OET. Among GLD-1 and CGH-1 co-regulated mRNAs, 34 mRNAs have a known function and of these 30 are required during OET. Proteins encoded by these 'OET mRNAs' are grouped according to when they are first required. (C) A model how GLD-1 and CGH-1 co-regulate mRNAs. GLD-1 binding mediates repression of translational initiation and primes transcripts for CGH-1 dependent mRNA stabilization. This leads to mRNA storage until transcripts are reactivated (translated) when they are needed in oocytes or the embryo. See also Table S2.

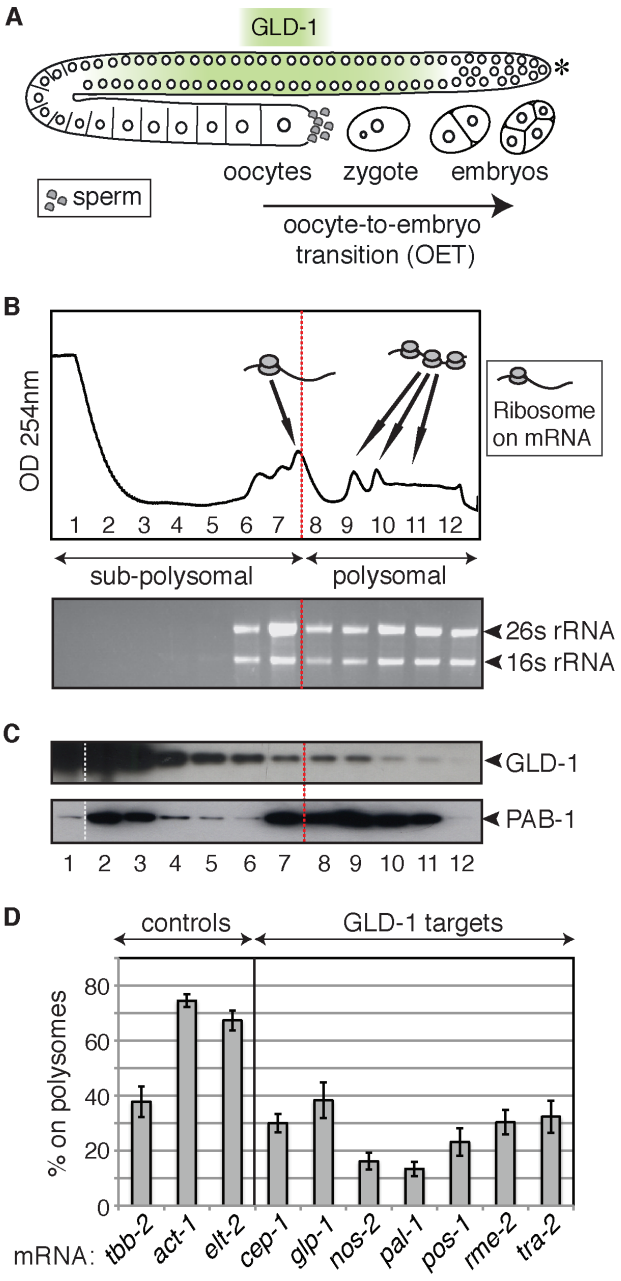

Fig. 1 Scheckel et al.

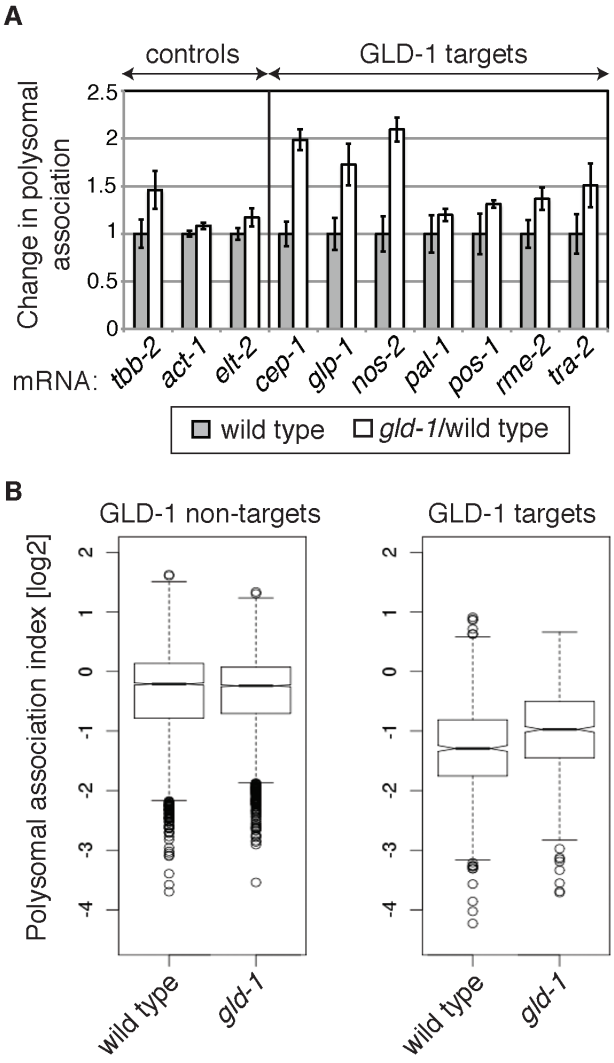

Fig. 2 Scheckel et al.

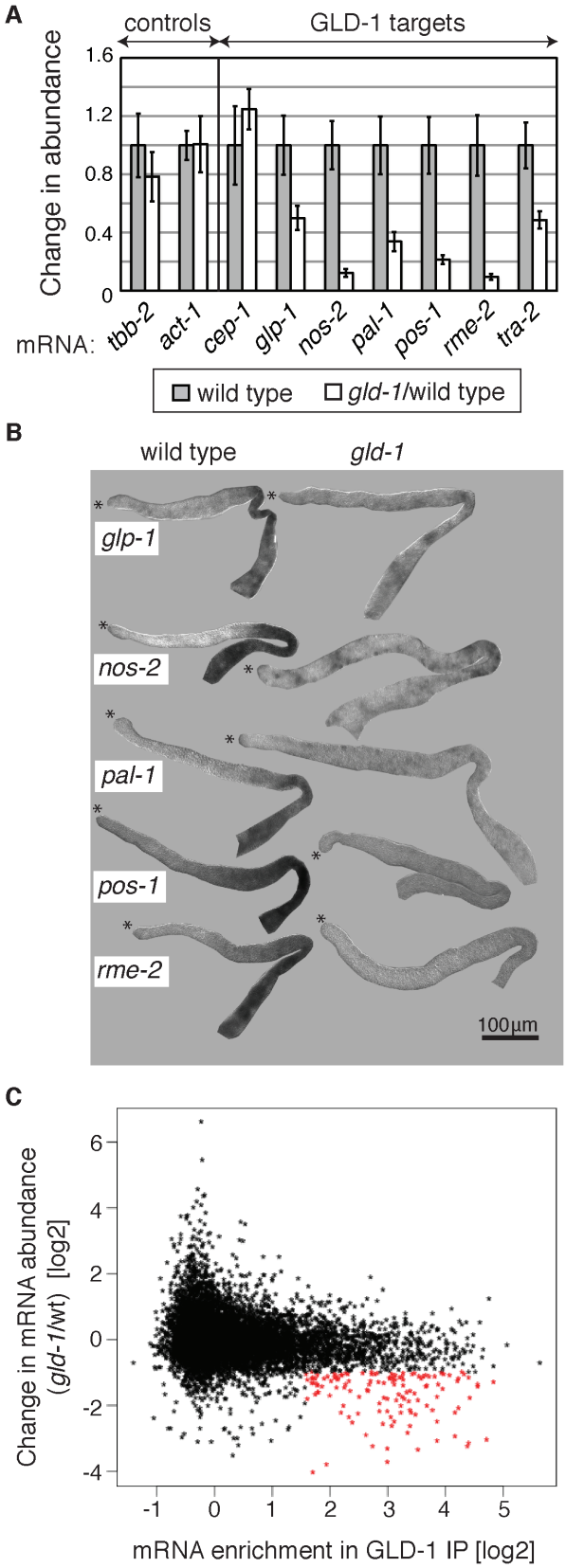

Fig. 3 Scheckel et al.

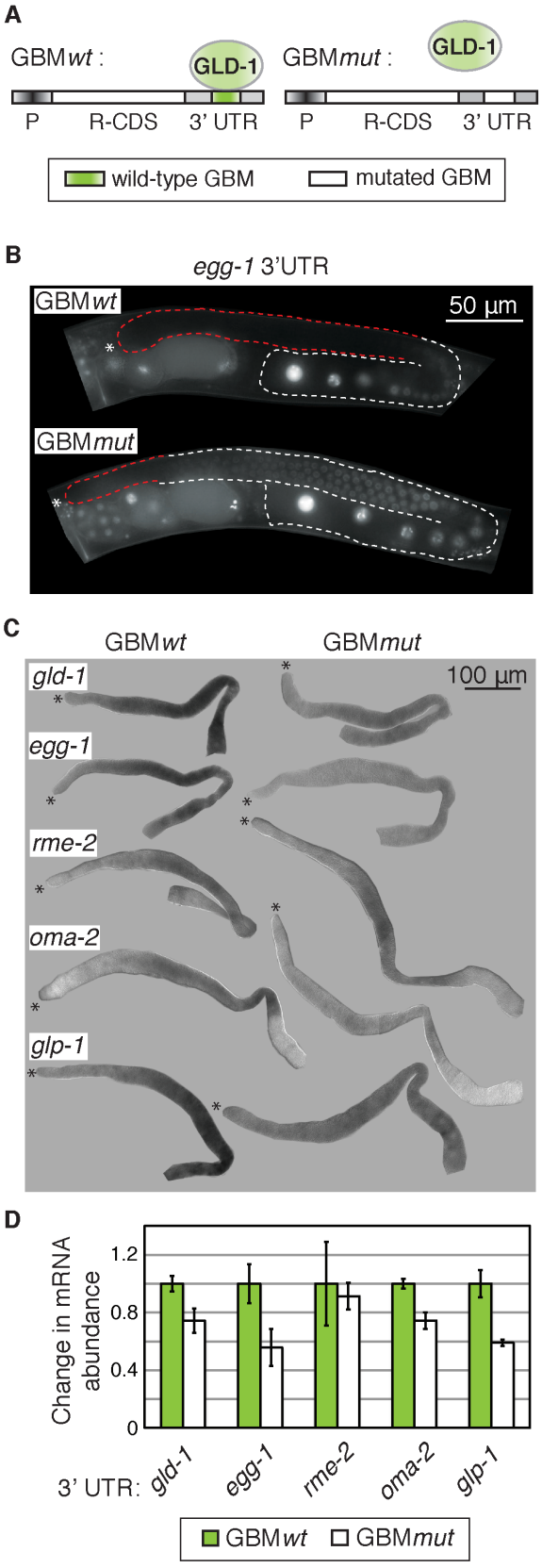

Fig. 4 Scheckel et al.

A

Mouse	Frog	Fly	Worm
RCK/p54	Xp54	Me31B	CGH-1
RAP55	RAP55	Tral	CAR-1
MSY-1	FRGY-2	Yps	CEY-1,-2,-3,-4
PABP	PABP	PABP	PAB-1
?	?	Sqd	SQD-1
Purα	?	Pur-alpha	PLP-1

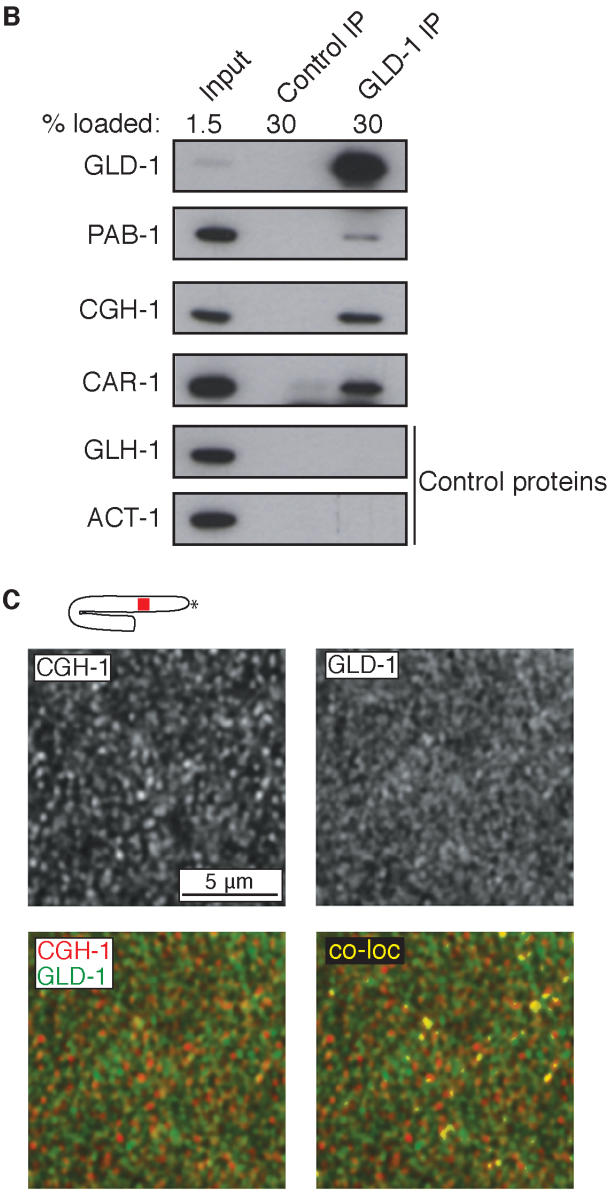

Fig. 5 Scheckel et al.

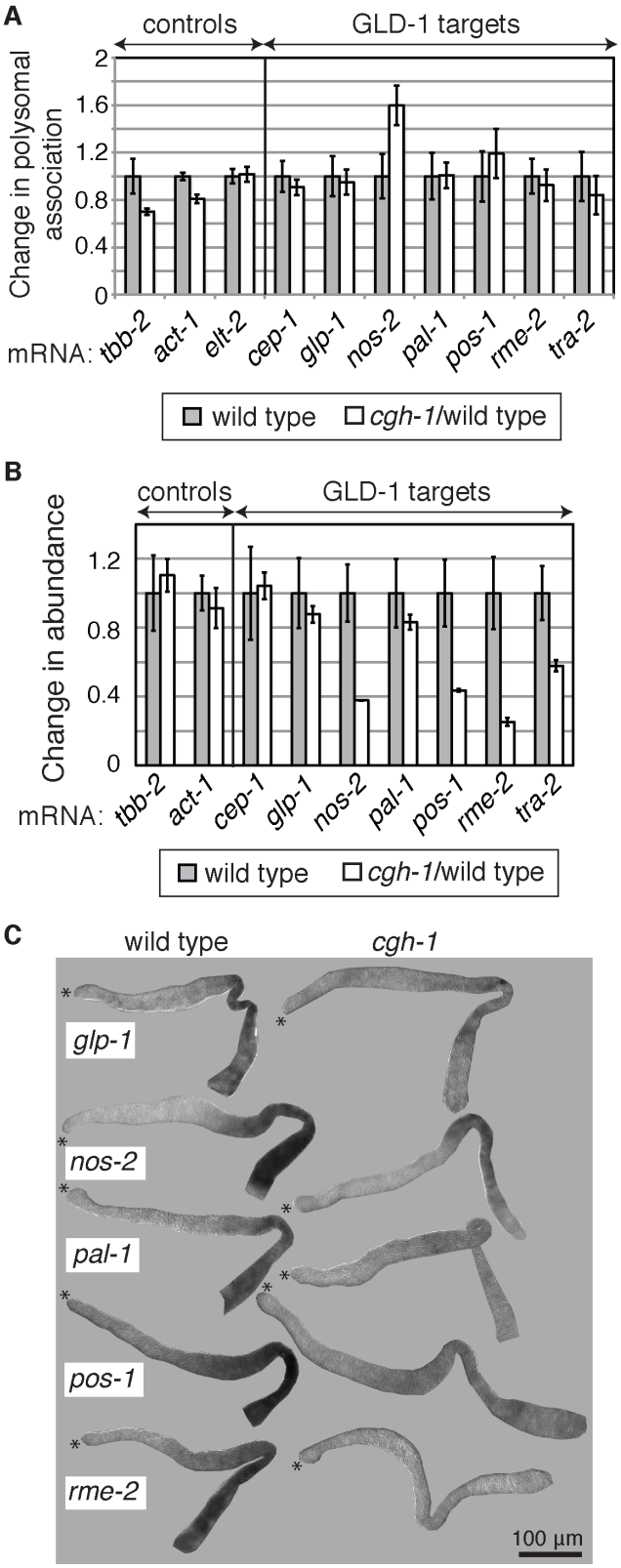

Fig. 6 Scheckel et al.

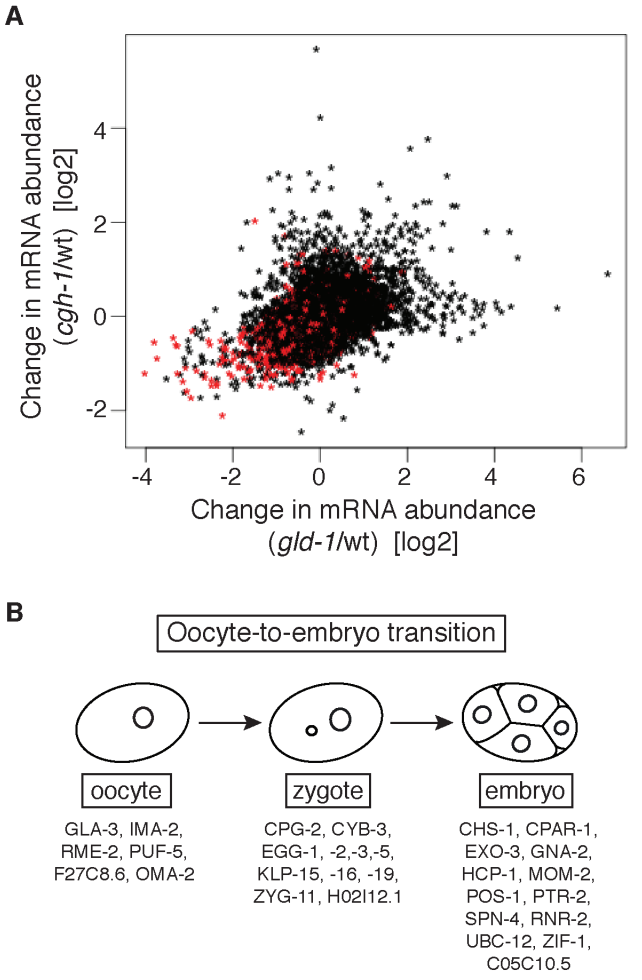

Fig. 7 Scheckel et al.

Supplemental Data

GLD-1 binding marks specific mRNA targets for accumulation in oocytes

Claudia Scheckel, Dimos Gaidatzis, Jane E. Wright, and Rafal Ciosk

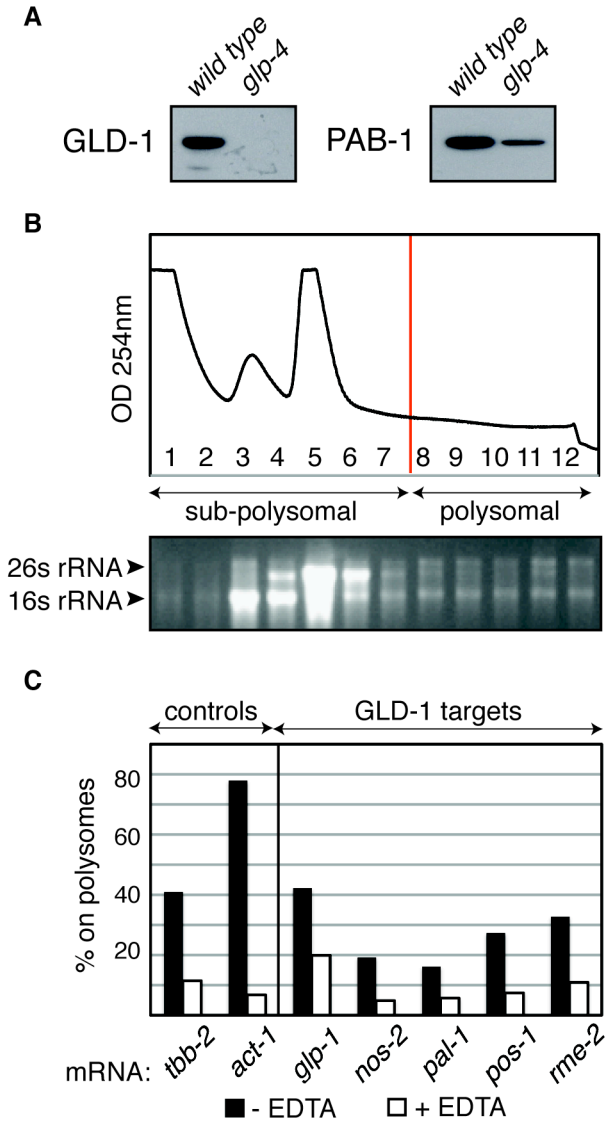

Figure S1, related to Figure 1

(A) GLD-1 and PAB-1 are predominantly present in the germ line. Total worm extracts of wild-type and germline-less mutant (*glp-4*) animals were analyzed by western blotting. GLD-1 is only expressed in the germ line, PAB-1 is germ line-enriched. (B) Polysome profile of EDTA-treated extracts from young adults.

Polysomes (fractions 8 – 12) and monosomes (fraction 7) are disrupted by EDTA treatment. The integrity of total RNA from individual fractions was confirmed on an agarose gel. (C) The polysomal association of mRNAs is EDTA-sensitive. RNA from each fraction was isolated, analyzed by reverse-transcription and quantitative PCR (RT-qPCR), and normalized to mouse RNA that was added to each fraction. Shown are polysomal associations (fractions 8 – 12), normalized to total RNA (fractions 1 – 12). The polysomal association of mRNAs decreases upon EDTA treatment, suggesting that mRNAs present in the heavy fractions are associated with polysomes and actively translated.

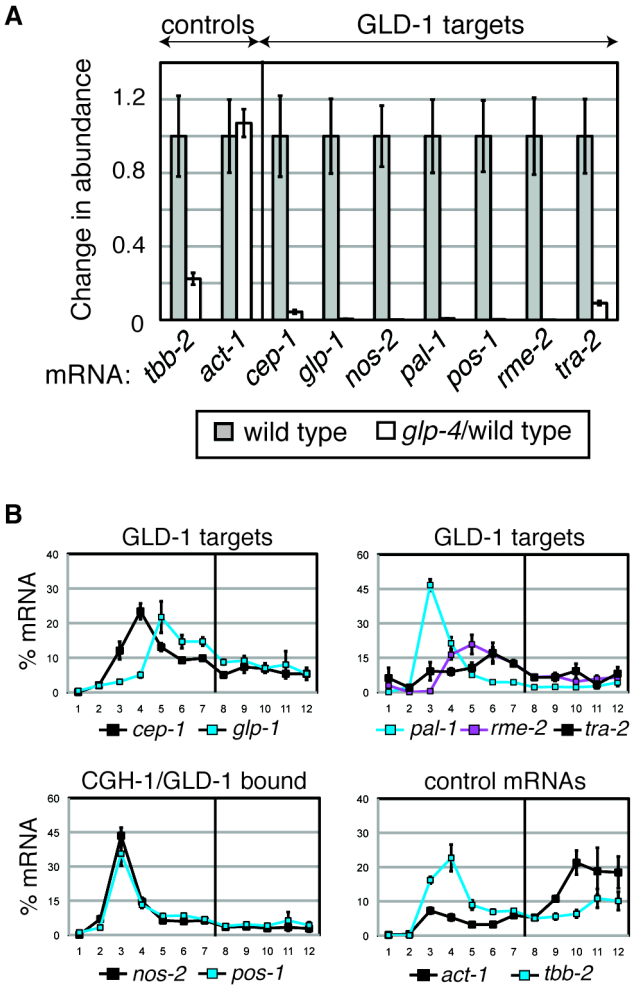

Figure S2, related to Figure 1

(A) Investigated mRNAs are predominantly expressed in the germ line. RT-qPCR quantification of indicted mRNAs from wild-type and germ line-less mutant (*glp-4*) animals. mRNA levels were normalized to a somatically expressed mRNA, *elt-2*, and to the wild-type levels. *tbb-2* is strongly enriched in the germline and is therefore also reduced in germline-less mutants. (B) GLD-1 targets are mostly present in sub-polysomal fractions. This is a detailed distribution of mRNA shown in Figure 1D. Compared to control mRNAs, GLD-1 targets are less associated with polysomes (fractions 8 – 12). Interestingly, different targets have different profiles, indicating that they might be present in distinct repressive complexes.

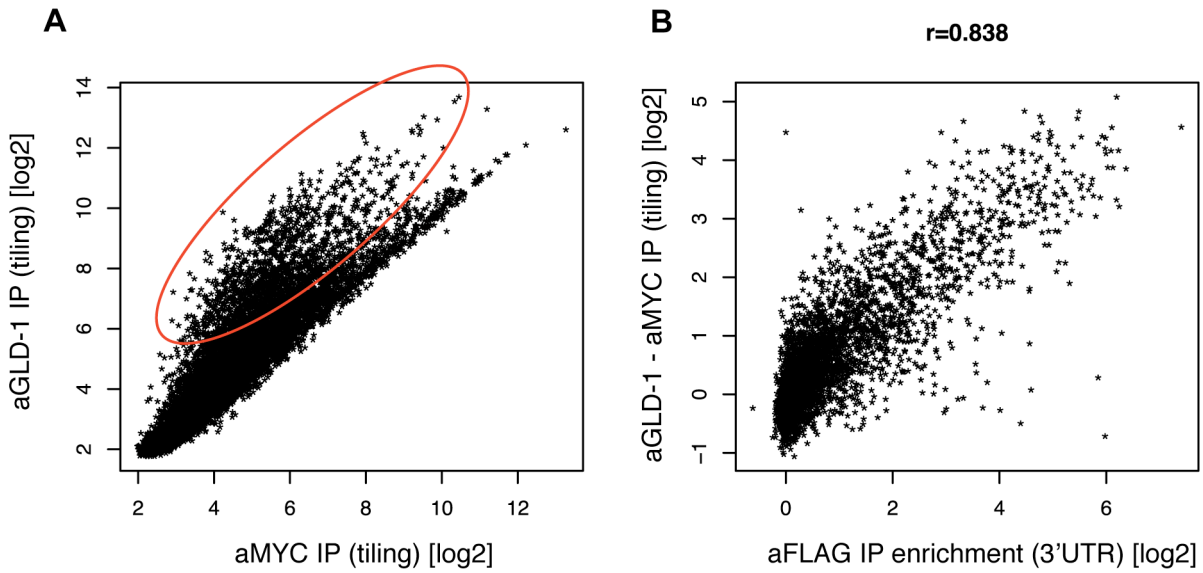

Figure S3, related to Figure 2

Identification of mRNAs associated with the endogenous GLD-1 protein. (A) 930 mRNAs (encircled) are enriched >3 fold in GLD-1 IPs, compared to control MYC IPs. Co-IPed mRNAs were analyzed by tiling arrays. Each dot represents a single transcript. (B) Similar mRNAs co-purify with the endogenous GLD-1 and a rescuing, FLAG and GFP-tagged GLD-1. mRNA enrichment in the FLAG-GFP-GLD-1 IP, measured by 3' Gene Expression Arrays, is shown on the X axis, as recently reported (Wright et al., 2010). The enrichment in the endogenous GLD-1 IPs, measured by tiling arrays, is shown on the Y axis. The correlation coefficient is 0.838, indicating that both the endogenous and tagged GLD-1 proteins interact with nearly identical mRNAs. Additional comparisons suggest that most differences result from using different microarray platforms (data not shown).

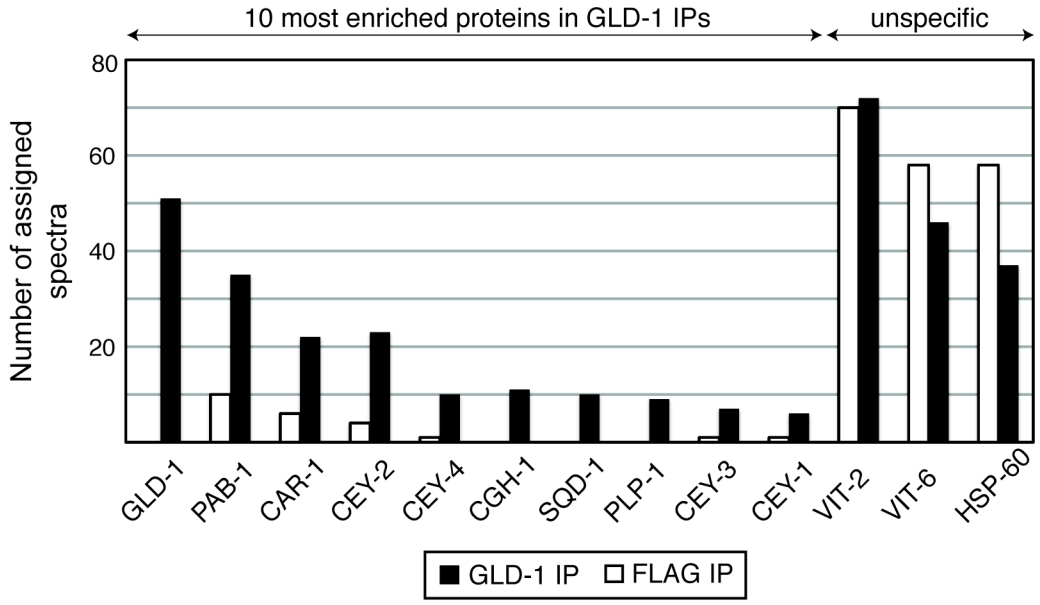

Figure S4, related to Figure 5

GLD-1 interacts with conserved RNA regulators. Proteins in the GLD and FLAG IPs were analyzed by mass spectrometry. Shown is the total number of assigned spectra of all peptides per protein. GLD-1 itself and a set of conserved RNA binding proteins were enriched in GLD-1 but not negative control (FLAG) IPs. Proteins such as VIT-2, VIT-6 and HSP-60 were equally enriched in both IPs.

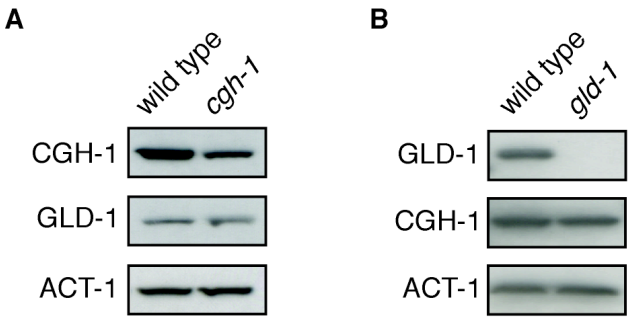

Figure S5, related to Figure 6

GLD-1 and CGH-1 do not influence protein levels of one another. (A) Loss of CGH-1 function does not affect GLD-1 protein levels. Total worm extracts from wild type and *cgh-1(tn691)* mutants were analyzed by western blotting. The temperature sensitive point mutant allele *tn691* only decreases CGH-1 levels at the restrictive temperature but phenocopies a null mutant allele, indicating that the remaining CGH-1 protein is not functional. (B) Loss of GLD-1 does not affect CGH-1 protein levels. Total worm extracts from wild-type and *gld-1(q485)* mutants were analyzed by western blotting. GLD-1 is not detectable, while CGH-1 and ACT-1 levels are not affected.

Table S1, related to Figure 2

All germline expressed genes and corresponding values for GLD-1 IP enrichment, mRNA level change in *gld-1* and *cgh-1* mutants. Excel spread sheet showing the gene public name, GLD-1 IP enrichment [log2], mRNA level change [log2] in *gld-1* mutants and *cgh-1* mutants. Positive values in GLD-1 IP enrichment indicate that transcrips are enriched in GLD-1 IPs. Negative values in mRNA level change columns indicate that transcripts are destabilized in *gld-1* or *cgh-1* mutants.

GO term	% of mRNA associated	PValue	Fold enrichment over germline mRNAs	Benjamini
Cytokinesis	22.92	2.23E-06	6.97	6.11E-04
Embryonic development ending in birth or egg hatching	91.67	8.98E-06	1.61	0.001229781
Cell division	22.92	5.80E-05	4.83	0.005280829
Cell cycle	27.08	3.54E-04	3.28	0.023943706
Cell cycle progress	22.92	0.0023972	3.04	0.123242194
M phase	18.75	0.00713437	3.08	0.278895259
Cell cycle phase	18.75	0.00770995	3.04	0.261370667
Microtubule-based process	12.50	0.01324772	4.14	0.366671854
Oogenesis	10.42	0.016673631	4.95	0.40064368
Chromosome segregation	12.50	0.026717871	3.46	0.490624642
Polysaccharide metabolic process	6.25	0.028572609	11.04	0.484134823
Microtubule-based movement	6.25	0.033872083	10.08	0.516300008
Meiotic chromosome segregation	10.42	0.050152751	3.51	0.634697754

Table S2, related to Figure 7

GO terms of mRNAs that associate with GLD-1 and are destabilized in both *gld-1* and *cgh-1* mutants. Distribution and relative enrichment of Gene Ontology (GO_FAT) terms were determined using the DAVID version 6.7 website (http://david/abcc.ncifcrf.gov:8080/). GLD-1 and CGH-1 co-regulated mRNAs that are bound by GLD-1 (n=89) were compared to germ line-expressed mRNAs (background; n=8025)

Oligo	Function	Sequence
egg-1_3'-UTR_fw	3'-UTR cloning	ggggacagctttcttgtacaaagtgggattt ctattgttcttgtattatgatc
egg-1_3'-UTR_rv	3'-UTR cloning	ggggacaactttgtataataaagttggttttt gctctagcgttaatatttg
gld-1_3'-UTR_fw	3'-UTR cloning	ggggacagctttcttgtacaaagtgggaa aagttcacatttataactcacac
gld-1_3'-UTR_rv	3'-UTR cloning	ggggacaactttgtataataaagttgaacat ttgaagaaatgaaaatgtttc
glp-1 f	3'-UTR cloning	ggggacagctttcttgtacaaagtgggata atctatttaattcattaatttttcatttattg
glp-1 r	3'-UTR cloning	ggggacaactttgtataataaagttgattcg aaaaacaaaaagccctcaagatactaaag aattacaattcattaaatgtttgcaagataag
rme-2 f	3'-UTR cloning	ggggacagctttcttgtacaaagtggcaat tctactacaaaattac
rme-2 r	3'-UTR cloning	Ggggacaactttgtataataaagttgctca catctcgattgtcat
MS_act-1_p1	qPCR act-1 fw	ctatgttccagccatccttcttgg
MS_act-1_p2	qPCR act-1 rv	tgatcttgatcttcatggttgatgg
cep-1_fw_new	qPCR cep-1 fw	attcgtttagaacgctcactc
cep-1_rv_new	qPCR cep-1 rv	gtgaggaatctttcaagtcct
mmCytcf	qPCR Cyt-c fw	ctctatttcaacccttactttccc
mmCytcr	qPCR Cyt-c rv	tcaacaacatcttgagaccca
elt-2_ fw_new	qPCR elt-2 fw	agtaaacggaggaatgatgtg
elt-2_ fw_new	qPCR elt-2 rv	ctgctctgaaggtatttcca
gfp exon spa f317	qPCR gfp fw	caagacacgtgctgaagtc
gfp exon spa r497	qPCR gfp rv	ttgaagttaactttgaattcc
glp-1_qPCR_fw_2	qPCR glp-1 fw	aagtctgatggtcgtatgtc
glp-1_qPCR_rv_2	qPCR glp-1 rv	cattctttgcgataacaccag
nos-2_RT_fw	qPCR nos-2 fw	tgattggttgaatgacagca
nos-2_RT_rv	qPCR nos-2 rv	gacagtatccacatccatattcac
MS_pal-1_p1	qPCR pal-1 fw	cgtatggtttactcggattatcaacgc
MS_pal-1_p2	qPCR pal-1 fw	cgacgatcctttgcacgcctatt
pos-1_RT_fw	qPCR pos-1 fw	cttccaatgaaccctcgtgg
pos-1_RT_rv	qPCR pos-1 rv	agtattagcgtgagctccac
MS_rme-2_p1	qPCR rme-2 fw	cagctcttgccatcgactacatc
MS_rme-2_p2	qPCR rme-2 rv	ctttaatgactcgcttcccaatc
tbb-2 f 495	qPCR tbb-2 fw	gctcattctcggttgtacca
tbb-2 r 696	qPCR tbb-2 rv	tggtgagggatacaagatgg
tra-2_fw_new	qPCR tra-2 fw	gtttgaaagtgaagttcgacac
tra-2_rv_1	qPCR tra-2 rv	aggagatgatcgtttgattgag
IK295	In situ gfp probe fw	atgagtaaaggagaagaacttttc
IK297	In situ gfp probe rv	taatacgactcactatagggacttttgtata gttcatccatgccatg
glp-1 left	In situ glp-1 probe fw	cgacatgtgcaaggattacg
glp-1 right	In situ glp-1 probe rv	taatacgactcactatagggacttcttgcat

antisense		ggatgtctggag
nos-2 left	In situ nos-2 probe fw	ttcggattcgtttgacagtg
nos-2 right antisense	In situ nos-2 probe rv	taatacgactcactatagggacttcgtcggttttcgaagtctc
pal-1_fw_is	In situ pal-1 probe fw	ccacatcctctgagagaaaagatgctgc
pal-1_rv_T7_in situ	In situ pal-1 probe rv	taatacgactcactatagggactcgtacacggacattattcgttccagg
pos-1 left	In situ pos-1 probe fw	tcgctttcacatcagtcagc
pos-1 right antisense	In situ pos-1 probe rv	taatacgactcactatagggacttgaggcacgaagagtgaatg
T7_Rme-2_fwd_North	In situ rme-2 probe fw	aaggaatttgattgcgggaacggg
T7_Rme-2_rv_North	In situ rme-2 probe rv	taatacgactcactatagggtggcacattgtgtatccactccga

Table S3

List of oligos used in this study. Shown are names, function and oligo sequences.

Additional oligos were used to create GBM*mut* 3'-UTRs.

Strain number	Strain name
# 716	rrrSi50 [mex-5 pro::PEST:GFP-H2B::egg-1 3'-UTR; unc-119(+)]II
# 717	rrrSi95 [mex-5 pro::PEST:GFP-H2B::egg-1 3'-UTR GBM mut; unc-119(+)]II
# 718	rrrSi107 [mex-5 pro::PEST:GFP-H2B::gld-1 3'-UTR; unc-119(+)]II
# 719	rrrSi113 [mex-5 pro::PEST:GFP-H2B::gld-1 3'-UTR GBM mut; unc-119(+)]II
# 688	rrrSi117 [mex-5 pro::PEST:GFP-H2B::glp-1 3'-UTR; unc-119(+)]II
# 684	rrrSi98 [mex-5 pro::PEST:GFP-H2B::glp-1 3'-UTR GBM mut; unc-119(+)]II
# 720	rrrSi39 [mex-5 pro::PEST:GFP-H2B::oma-2 3'-UTR; unc-119(+)]II
# 721	rrrSi53 [mex-5 pro::PEST:GFP-H2B::oma-2 3'-UTR GBM mut; unc-119(+)]II
# 693	rrrSi122 [mex-5 pro::PEST:GFP-H2B::rme-2 3'-UTR; unc-119(+)]II
# 681	rrrSi96 [mex-5 pro::PEST:GFP-H2B::rme-2 3'-UTR GBM mut; unc-119(+)]II

Table S4

List of transgenic strains created in this study.

Supplemental References

Wright, J.E., Gaidatzis, D., Senften, M., Farley, B.M., Westhof, E., Ryder, S.P., and Ciosk, R. (2010). A quantitative RNA code for mRNA target selection by the germline fate determinant GLD-1. The EMBO Journal advance online publication, 17 Dec 2010. DOI 10.1038/emboj.2010.334

3.3. GLD-1-mediated mRNA stabilization in the germ line correlates with maternal mRNA destabilization in the embryo

3.3.1. Introduction

In the *C. elegans* early embryo, the germ line is set aside from the soma by a series of asymmetric cell divisions. The germline blastomere inherits germline-specific RNA granules, P granules, which contain various RBPs and mRNAs. The onset of zygotic transcription at the four-cell stage correlates with maternal mRNA degradation but importantly both events are limited to somatic blastomeres (Fig. 1).

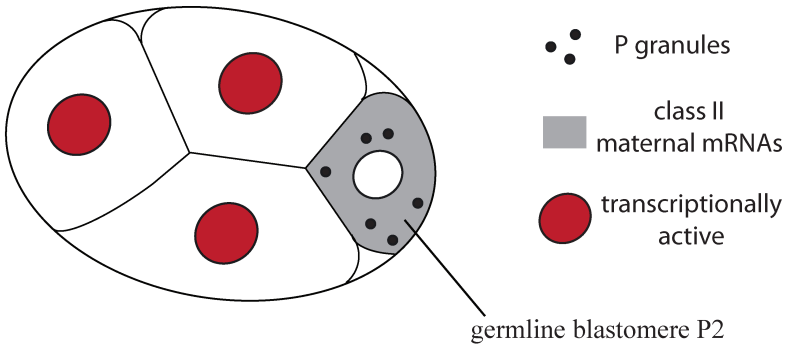

Figure 1
Onset of zygotic transcription and maternal mRNA degradation in the early embryo in *C. elegans*.
The 4-cell stage is the onset of zygotic transcription in *C. elegans*. Transcription is activated in somatic blastomeres but is prevented in the germline blastomere by PIE-1. At the same time, maternal class II mRNAs are degraded in somatic blastomeres but are retained in the germline blastomere.

A key player in the specification of germline blastomeres is the zinc finger protein PIE-1 (Mello et al. 1992; Mello et al. 1996; Seydoux et al. 1996). PIE-1 is excluded from somatic blastomeres due to the active segregation of PIE-1 to the germline precursors. Additionally PIE-1 and other germline proteins are degraded in the soma via ZIF-1, an E3 ubiquitin ligase subunit (Reese et al. 2000; DeRenzo et al. 2003). PIE-1 then inhibits transcription in germline blastomeres by mimicking a non-phosphorylatable form of the CTD of RNA Pol II and competing with the CTD for cyclin T binding. The CDK-9/cyclin T complex, also known as P-TEFb, can therefore no longer phosphorylate the CTD and transcription elongation is prevented (Seydoux and Dunn 1997; Batchelder et al. 1999; Zhang et al. 2003). So while transcription is

limited to somatic blastomeres in the early embryo, P granules and germline proteins are restricted to germline precursors. At the same time a set of maternal mRNAs is specifically degraded in the soma. In *C. elegans*, maternal mRNAs can be divided into two groups. While class I maternal mRNAs are present throughout the embryo, class II maternal mRNAs are asymmetrically distributed and are specifically maintained in germline blastomeres (Seydoux and Fire 1994). Before the onset of zygotic transcription and maternal mRNA degradation at the 4-cell stage, the expression of only a few mRNA changes (Fig. 2A).

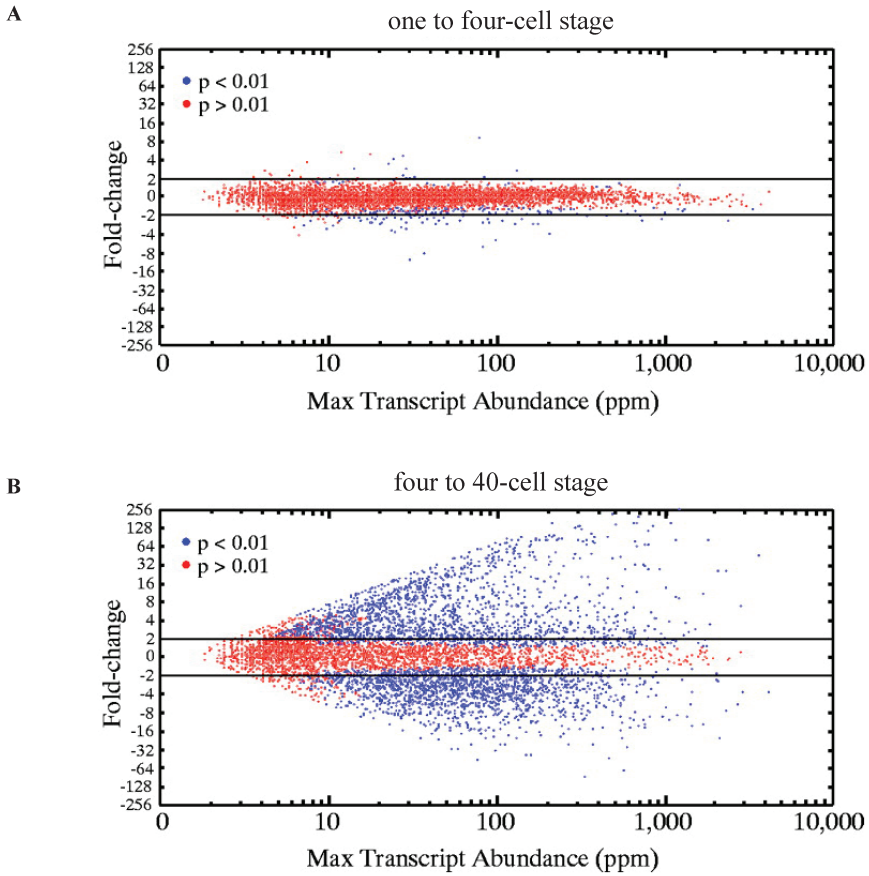

Figure 2
mRNAs levels are stable until the 4-cell stage, the onset of zygotic transcription and maternal mRNA degradation (adapted from Baugh et al., 2003, Fig.4).
Scatter plots of 8890 reproducibly detected genes. (A) The change in mRNA abundance that oocurs between the one and four cells stage is plotted on the y-axis on a log2 scale. The max of the two mean transcript abundances is plotted on the x-axis on a log10 scale. The two lines crossing the y-axis at +/- 2 mark twofold changes. Each point is color coded according to whether or not the observed difference is statistically significant ($P < 0.01$) according to paired-timepoint ANOVA. The number of genes that are considered to be significantly different is 217, 38 of which show an increase and 179 show a decrease. (B) Changes in transcript abundance that occur between the four and fourty cell stage are plotted on the y-axis on a log2 scale. The plot is otherwise identical to B. Of the 3773 genes that are considered significantly different, 1911 show an increase and 1862 show a decrease.

In contrast, after the 4-cells stage the abundance of the majority of transcripts changes dramatically, either mRNAs are upregulated due to zygotic transcription or they are downregulated due to maternal mRNA degradation (Fig. 2B) (Baugh et al. 2003).

As previously discussed, GLD-1 is key regulator of translational regulation in the *C. elegans* germ line. Interestingly, GLD-1 is also expressed in posterior blastomeres of the embryo (including the germline precursor) (Jones et al. 1996). The role of GLD-1 during embryogenesis is not well understood. Homozygous mutant animals derived from a heterozygous parent develop normally and only develop a germline tumor during adulthood. On the other hand these homozygous mutants inherit maternally transcribed *gld-1*, which might be sufficient to recapitulate GLD-1 expression in the embryo. In addition, it has been shown that GLD-1 represses the translation of its target *glp-1* (encoding for the Notch receptor) not only in mitotically dividing cells in the germ line but also in posterior blastomeres of the embryo. Moreover the embryos that showed loss of GLD-1 mediated *glp-1* repression did not produce any offspring (Marin and Evans 2003). This suggests that GLD-1 mediated mRNA regulation might also be important for embryogenesis.

Although it has been known for almost 20 years that specific mRNAs, class II maternal mRNAs, are targeted to undergo mRNA decay in the early embryo it is still unclear how these mRNAs are targeted for degradation and what the mechanisms mediating this degradation are. We analyzed class II maternal mRNAs in more detail and found that GLD-1 stabilized target mRNAs are preferentially degraded in the early embryo. This suggests that these GLD-1 targets are not only marked for stabilization in the germ line but also for degradation in the embryo.

3.3.2. Results

To discriminate between maternal and zygotic mRNA degradation during embryogenesis we compared the mRNA expression between two very early time points, which have been published previously (Baugh et al. 2003). Several mRNAs increase in their expression level from the 4 to 16-cell stage due to zygotic

transcription (encircled in green in Fig. 3). At the same time the abundance of many transcripts decreases from the 4 to 16-cell stage (encircled in red). Since zygotic transcription only starts at the 4-cell stage these mRNAs are largely class II maternal mRNAs.

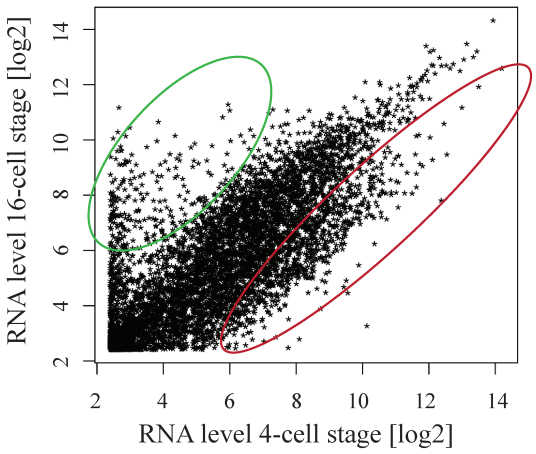

Figure 3
Many mRNAs are degraded between the 4 and 16 cell stage of the embryo.
Comparison of the mRNA level between 4 and 16 cell stage embryos. Maternal class II mRNAs are encircled in red whereas zygotically transcribed mRNAs are encircled in green. Maternal class I mRNAs are represented as dots on the diagonal and don't change between the two stages.

It is currently unclear how mRNAs are degraded in the embryo and importantly how class II maternal mRNAs are specifically marked for degradation while class I maternal mRNAs continue to be expressed throughout the embryo.

Since GLD-1 is not only expressed in the germ line but also in posterior blastomeres of the embryo, we wondered if GLD-1 mediated mRNA regulation might be connected to maternal mRNA degradation (Fig. 4). We grouped mRNAs according to three criteria: (1) mRNAs that are bound by GLD-1 (2) mRNAs that are stabilized by GLD-1 (destabilized in *gld-1* mutants) and (3) mRNAs that are stabilized by CGH-1 (destabilized in *cgh-1* mutants). It is of note that mRNAs stabilized by either GLD-1 or CGH-1 are not necessarily direct targets of these proteins but are termed here as such for simplicity.

mRNAs that fulfill only one of these criteria only show a small tendency to get degraded in the embryo (comparing white with grey boxes). Also mRNAs that are stabilized by CGH-1 and either bound by GLD-1 or stabilized by GLD-1 behave like the control group (comparing white with grey boxes). Interestingly, mRNAs that are

both GLD-1 bound and stabilized show a clear trend to undergo maternal mRNA degradation (comparing white with red boxes). In contrast, CGH-1 mediated stabilization has only a minor contribution to determine class II maternal mRNAs (comparing both red boxes).

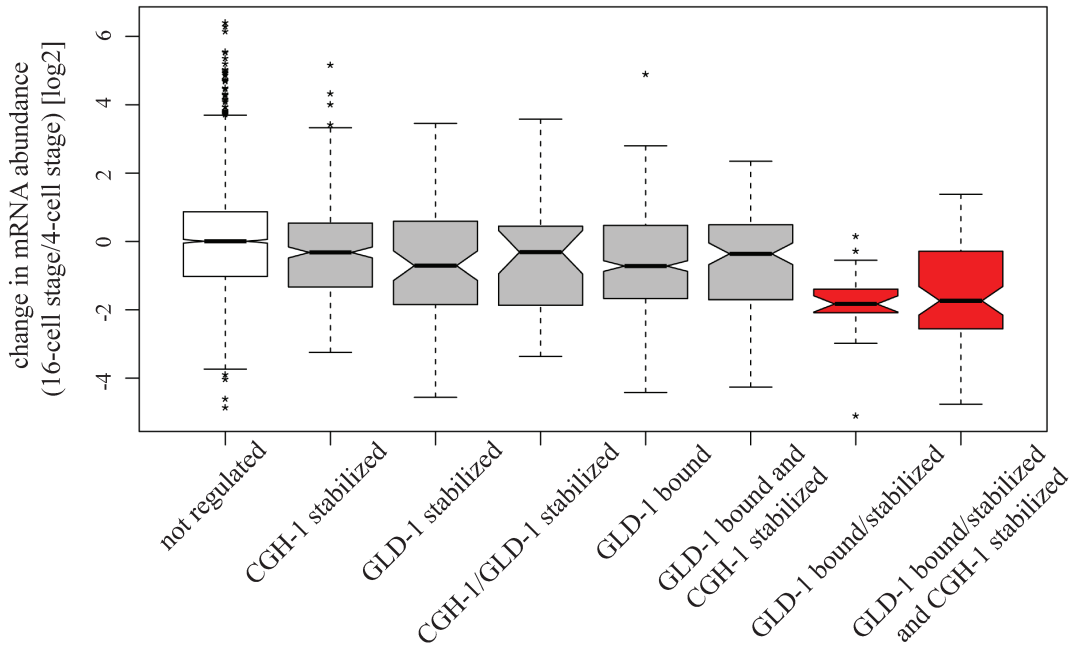

Figure 4
GLD-1 mediated mRNA stabilization correlates with maternal mRNA degradation.
mRNAs were grouped into categories depending on the following criteria: "CGH-1 stabilized", "GLD-1 stabilized" and "GLD-1 binding". The mRNA level change between 4- and 16-cell stage embryos is plotted on the y-axis in log2 scale. Negative values indicate that mRNAs are degraded from the 4 to the 16-cell stage. Only mRNAs that are bound AND stabilized by GLD-1 tend to get degraded in the embryo and are displayed as red boxes. In contrast, CGH-1-mediated stabilization does not contribute to this trend (no difference in maternal mRNA abundance between the two red boxes).

This suggests that GLD-1 mediated mRNA stabilization (GLD-1 binding and stabilization) contributes to determining which mRNAs undergo maternal mRNA degradation in the embryo.

3.3.3. Discussion

Several cis-acting elements and trans-acting factors that mediate the clearance of maternal mRNAs have been identified in *Drosophila*. Among the trans-acting factors are the RBPs Smaug as well as miRNAs and piRNAs (Rouget et al. 2010; Thomsen et

al. 2010; Tadros et al. 2007; Bushati et al. 2008). Although maternal mRNA degradation has been globally analyzed in *C. elegans*, no RNA sequences or trans-acting factors mediating this process have been identified.

Analysis of maternal mRNA degradation with respect to GLD-1 and CGH-1 mediated mRNA regulation revealed that GLD-1 plays a role in determining class II maternal mRNAs. mRNAs that are bound and stabilized by GLD-1 in the germ line tend to undergo maternal mRNA degradation in the embryo. Several models are conceivable to explain this observation.

On one hand, GLD-1 binding in the germ line might already influence the fate of the mRNA in the embryo. GLD-1 could recruit additional factors only to mRNAs it stabilizes. These proteins could then remain bound to the GLD-1 stabilized mRNAs and target mRNAs for maternal mRNA degradation in the somatic blastomeres by interacting with mRNA decay enzymes. Or alternatively, these proteins might get degraded in the soma thereby subjecting previously bound mRNAs to degradation while the protective RNP complex persists in germline precursors.

In another model, class II maternal mRNAs are marked by GLD-1 not in the germ line but only in the embryo. In germline blastomeres GLD-1 might not only prevent the expression of its targets as shown for *glp-1* (Marin and Evans 2003) but also prevent their degradation. This would then suggest that GLD-1 binding not only marks specific mRNA targets for accumulation in oocytes but also in germline precursors.

3.3.4. Experimental Procedures

Experimental procedures are described in section 3.2.

4. General discussion and outlook

The importance of post-transcriptional regulation has been implicated in virtually every biological process. For example in the brain, many events such as axon guidance, dendritic morphogenesis and synaptic plasticity depend on local protein synthesis. Many RNA regulators have been identified in neurons and accordingly, several neurological disorders have been linked to RBPs (Sutton and Schuman 2006). Translational control is also a crucial part of cancer development. The proliferation of cancer cells depends on an increase in mRNA translation and the misregulation of numerous translation factors and regulatory proteins is associated with disease progression (Silvera et al. 2010).

Also Quaking-related (QR) proteins act as tumor suppressors by preventing cell proliferation and promoting differentiation (Biedermann et al. 2010). Similarly in the *C. elegans* germ line, the QR-protein GLD-1 inhibits mitotic proliferation and stimulates oogenesis, the equivalent of differentiation in the germ line. GLD-1 binds and regulates many mRNAs, which is crucial for germ cell development. Loss of this regulation not only prevents oogenesis but leads to the formation of a proliferative tumor. Moreover, cells transdifferentiate and adopt somatic cells fates, revealing an additional role of GLD-1 in maintaining germ cell identity (Ciosk et al. 2006). GLD-1 associates with numerous mRNAs and globally represses the translation of these transcripts by interfering with translation initiation. Additionally, GLD-1 also stabilizes a large group of its targets, suggesting that GLD-1 plays a central role in mediating maternal mRNA storage.

The finding that GLD-1 globally represses translation initiation is consistent with our observation that many germ line mRNAs are mostly associated with sub-polysomal fractions. While several lines of evidence suggest that translational repression is prominent in the germ line, we show directly that many mRNAs are specifically repressed in the germ line. Interestingly, the translational machinery gets targeted for repression specifically in the soma, while overall only little mRNA repression can be observed in the soma. We therefore conclude that the germ line is the main compartment of translational repression in *C. elegans*.

At this point we don't know how GLD-1 prevents translation initiation. We couldn't detect an interaction between GLD-1 and any translation initiation factors or the *C. elegans* 4E-BP, SPN-2, suggesting that GLD-1 might not directly interfere with the assembly of the translation initiation complex. Alternatively, GLD-1 could prevent translation initiation by sequestering mRNAs away from the translational machinery. A similar process has been described as mRNA masking, and for example in *Drosophila* oocytes *oskar* is assembled into silencing particles that cannot get accessed by ribosomes (Chekulaeva et al. 2006). Along those lines, GLD-1 interacts with several RBPs that are components of repressive RNPs in various species, including the DDX6 helicase CGH-1.

CGH-1 has previously been implicated in stabilizing some mRNAs in the *C. elegans* germline. Importantly, CGH-1 and GLD-1 protect common mRNAs, whereas GLD-1 mediated translational repression can occur independently of CGH-1. The only partly overlapping function of both proteins is consistent with *gld-1* and *cgh-1* mutants showing different phenotypes. Among other things, GLD-1 promotes meiotic entry and progression, and loss of GLD-1 leads to the formation of a proliferative tumor and transdifferentiation. In contrast, CGH-1 is required for gametogenesis and *cgh-1* mutant gonads still contain (defective) oocytes. Whereas apoptosis is inhibited in *gld-1* mutants, apoptosis is elevated in *cgh-1* mutants. We therefore sought to determine the consequences of inhibiting apoptosis in *cgh-1* mutants. However, when we analyzed *cgh-1; ced-3* double mutants (with ced-3 encoding a protease required for apoptosis), we found that these worms were both, phenotypically and regarding mRNA stabilization, very similar to *cgh-1* single mutant worms (data not shown). This suggests the difference in apoptosis in *gld-1* and *cgh-1* mutants cannot account for the different mutant phenotypes.

Since GLD-1 and CGH-1 seem to reside largely in separate cytoplasmic foci, we propose that the two proteins induce mRNA storage in a step-wise model. GLD-1 initially associates with the 3' UTRs of its targets through GBMs. This allows CGH-1, which by itself has little RNA binding specificity, to target these transcripts for stabilization and storage. Interestingly GLD-1 and CGH-1 only stabilize a subset of GLD-1 targets, suggesting that CGH-1 is only recruited to certain GLD-1 targets. To date we don't know why only certain transcripts are bound by CGH-1 and importantly

how CGH-1 binds these transcripts. Preliminary data suggests that the interaction between GLD-1 and CGH-1 is RNA dependent, indicating that an additional co-factor might determine which mRNAs associate with CGH-1 and are ultimately targeted for mRNA storage. However, it seems that translational repression can occur independently of mRNA stabilization.

At this point we also don't know where GLD-1 and CGH-1 protect mRNAs from the decay machinery. CGH-1 is expressed in meiotic germ cells, i.e. in the central and proximal germ line, and the early embryo. In contrast GLD-1 is only present in the central gonad. GLD-1 is therefore no longer expressed where many of its targets still need to be stored in a repressed and stable form. One possibility is that mRNAs are only targeted for stabilization in early meiotic cells, where GLD-1 is present. This stabilization might be sufficient for mRNA accumulation in oocytes, for example because mRNAs might no longer get degraded in oocytes. Alternatively, GLD-1 is not required for the maintenance but only for the initiation of maternal mRNA storage, consistent with our step-wise model of maternal mRNA storage. Other proteins such as CGH-1 would then ensure that mRNAs continue to be repressed and stabilized until they are needed.

During embryogenesis, cells become again transcriptionally active. The embryo no longer relies solely on maternal mRNAs but becomes dependent on zygotic transcripts. With the onset of zygotic gene activation, maternal mRNAs also start to get degraded. The importance of maternal mRNA degradation is highlighted in *Drosophila*, where a mutant defective for maternal mRNA clearance is also defective for zygotic gene activation (Benoit et al. 2009). In *C. elegans* only certain maternal mRNAs undergo decay at the 4-cell stage of embryogenesis and while they get cleared in the soma they are retained in germ line precursors. It is unknown how these class II maternal mRNAs are degraded and importantly what targets them for degradation. To date neither specific sequences nor trans-acting factors have been identified with respect to maternal mRNA decay. Interestingly, we found that mRNAs targeted for GLD-1 dependent storage are not only subject to stabilization in the germ line but also to degradation in somatic blastomeres in the embryo. To date we don't know if this reflects a novel function of GLD-1 in the embryo or whether these

mRNAs are already marked for maternal mRNA degradation in the germ line. Class II maternal mRNAs might have intrinsic characteristics that targets them for degradation. Since GLD-1 is expressed in germline precursors in the embryo, the decay machinery might not have access to GLD-1 bound mRNAs. Alternatively, trans-acting factors that have been deposited on transcripts in a GLD-1-dependent manner in the germ line might still protect these mRNAs in germline blastomeres. Yet another possibility is that these factors recruit mRNA decay enzymes specifically in somatic blastomeres. Concluding, while we found a correlation between GLD-1 mediated mRNA stabilization and class II maternal mRNA degradation, it remains unclear how this causal relationship is established.

To sum up, we observed that the translation initiation of numerous mRNAs is repressed in the germ line and that GLD-1 plays a central role in preventing translation initiation of many mRNAs. Moreover, GLD-1 binding also marks certain mRNAs for CGH-1 dependent mRNA stabilization and additionally for maternal mRNA degradation in the embryo. These findings are consistent with and emphasize the role of GLD-1 as a key regulator of the *C. elegans* germ line.

References

Alkalaeva, E.Z., Pisarev, A.V., Frolova, L.Y., Kisselev, L.L., and Pestova, T.V. 2006. In vitro reconstitution of eukaryotic translation reveals cooperativity between release factors eRF1 and eRF3. *Cell* **125**(6): 1125-1136.

Andrei, M.A., Ingelfinger, D., Heintzmann, R., Achsel, T., Rivera-Pomar, R., and Luhrmann, R. 2005. A role for eIF4E and eIF4E-transporter in targeting mRNPs to mammalian processing bodies. *RNA* **11**(5): 717-727.

Andrews, S., Snowflack, D.R., Clark, I.E., and Gavis, E.R. 2011. Multiple mechanisms collaborate to repress nanos translation in the Drosophila ovary and embryo. *RNA* **17**(5): 967-977.

Aroian, R.V., Levy, A.D., Koga, M., Ohshima, Y., Kramer, J.M., and Sternberg, P.W. 1993. Splicing in Caenorhabditis elegans does not require an AG at the 3' splice acceptor site. *Mol Cell Biol* **13**(1): 626-637.

Austin, J. and Kimble, J. 1987. glp-1 is required in the germ line for regulation of the decision between mitosis and meiosis in C. elegans. *Cell* **51**(4): 589-599.

Bachorik, J.L. and Kimble, J. 2005. Redundant control of the Caenorhabditis elegans sperm/oocyte switch by PUF-8 and FBF-1, two distinct PUF RNA-binding proteins. *Proc Natl Acad Sci U S A* **102**(31): 10893-10897.

Barnard, D.C., Cao, Q., and Richter, J.D. 2005. Differential phosphorylation controls Maskin association with eukaryotic translation initiation factor 4E and localization on the mitotic apparatus. *Mol Cell Biol* **25**(17): 7605-7615.

Barnard, D.C., Ryan, K., Manley, J.L., and Richter, J.D. 2004. Symplekin and xGLD-2 are required for CPEB-mediated cytoplasmic polyadenylation. *Cell* **119**(5): 641-651.

Bashirullah, A., Halsell, S.R., Cooperstock, R.L., Kloc, M., Karaiskakis, A., Fisher, W.W., Fu, W., Hamilton, J.K., Etkin, L.D., and Lipshitz, H.D. 1999. Joint action of two RNA degradation pathways controls the timing of maternal transcript elimination at the midblastula transition in Drosophila melanogaster. *EMBO J* **18**(9): 2610-2620.

Batchelder, C., Dunn, M.A., Choy, B., Suh, Y., Cassie, C., Shim, E.Y., Shin, T.H., Mello, C., Seydoux, G., and Blackwell, T.K. 1999. Transcriptional repression by the Caenorhabditis elegans germ-line protein PIE-1. *Genes Dev* **13**(2): 202-212.

Baugh, L.R., Hill, A.A., Slonim, D.K., Brown, E.L., and Hunter, C.P. 2003. Composition and dynamics of the Caenorhabditis elegans early embryonic transcriptome. *Development* **130**(5): 889-900.

Benoit, B., He, C.H., Zhang, F., Votruba, S.M., Tadros, W., Westwood, J.T., Smibert, C.A., Lipshitz, H.D., and Theurkauf, W.E. 2009. An essential role for the RNA-binding protein Smaug during the Drosophila maternal-to-zygotic transition. *Development* **136**(6): 923-932.

Benoit, P., Papin, C., Kwak, J.E., Wickens, M., and Simonelig, M. 2008. PAP- and GLD-2-type poly(A) polymerases are required sequentially in cytoplasmic

polyadenylation and oogenesis in Drosophila. *Development* **135**(11): 1969-1979.

Besse, F., Lopez de Quinto, S., Marchand, V., Trucco, A., and Ephrussi, A. 2009. Drosophila PTB promotes formation of high-order RNP particles and represses oskar translation. *Genes Dev* **23**(2): 195-207.

Biedermann, B., Hotz, H.R., and Ciosk, R. 2010. The Quaking family of RNA-binding proteins: coordinators of the cell cycle and differentiation. *Cell Cycle* **9**(10): 1929-1933.

Biedermann, B., Wright, J., Senften, M., Kalchhauser, I., Sarathy, G., Lee, M.H., and Ciosk, R. 2009. Translational repression of cyclin E prevents precocious mitosis and embryonic gene activation during C. elegans meiosis. *Dev Cell* **17**(3): 355-364.

Boag, P.R., Atalay, A., Robida, S., Reinke, V., and Blackwell, T.K. 2008. Protection of specific maternal messenger RNAs by the P body protein CGH-1 (Dhh1/RCK) during Caenorhabditis elegans oogenesis. *J Cell Biol* **182**(3): 543-557.

Boag, P.R., Nakamura, A., and Blackwell, T.K. 2005. A conserved RNA-protein complex component involved in physiological germline apoptosis regulation in C. elegans. *Development* **132**(22): 4975-4986.

Bobola, N., Jansen, R.P., Shin, T.H., and Nasmyth, K. 1996. Asymmetric accumulation of Ash1p in postanaphase nuclei depends on a myosin and restricts yeast mating-type switching to mother cells. *Cell* **84**(5): 699-709.

Breitwieser, W., Markussen, F.H., Horstmann, H., and Ephrussi, A. 1996. Oskar protein interaction with Vasa represents an essential step in polar granule assembly. *Genes Dev* **10**(17): 2179-2188.

Bushati, N., Stark, A., Brennecke, J., and Cohen, S.M. 2008. Temporal reciprocity of miRNAs and their targets during the maternal-to-zygotic transition in Drosophila. *Curr Biol* **18**(7): 501-506.

Bushman, J.L., Asuru, A.I., Matts, R.L., and Hinnebusch, A.G. 1993. Evidence that GCD6 and GCD7, translational regulators of GCN4, are subunits of the guanine nucleotide exchange factor for eIF-2 in Saccharomyces cerevisiae. *Mol Cell Biol* **13**(3): 1920-1932.

Caceres, L. and Nilson, L.A. 2009. Translational repression of gurken mRNA in the Drosophila oocyte requires the hnRNP Squid in the nurse cells. *Dev Biol* **326**(2): 327-334.

Cao, Q., Padmanabhan, K., and Richter, J.D. 2010. Pumilio 2 controls translation by competing with eIF4E for 7-methyl guanosine cap recognition. *RNA* **16**(1): 221-227.

Caput, D., Beutler, B., Hartog, K., Thayer, R., Brown-Shimer, S., and Cerami, A. 1986. Identification of a common nucleotide sequence in the 3'-untranslated region of mRNA molecules specifying inflammatory mediators. *Proc Natl Acad Sci U S A* **83**(6): 1670-1674.

Chekulaeva, M., Hentze, M.W., and Ephrussi, A. 2006. Bruno acts as a dual repressor of oskar translation, promoting mRNA oligomerization and formation of silencing particles. *Cell* **124**(3): 521-533.

Chen, J., Melton, C., Suh, N., Oh, J.S., Horner, K., Xie, F., Sette, C., Blelloch, R., and Conti, M. 2011. Genome-wide analysis of translation reveals a critical role for deleted in azoospermia-like (Dazl) at the oocyte-to-zygote transition. *Genes Dev* **25**(7): 755-766.

Chen, T., Damaj, B.B., Herrera, C., Lasko, P., and Richard, S. 1997. Self-association of the single-KH-domain family members Sam68, GRP33, GLD-1, and Qk1: role of the KH domain. *Mol Cell Biol* **17**(10): 5707-5718.

Cho, P.F., Gamberi, C., Cho-Park, Y.A., Cho-Park, I.B., Lasko, P., and Sonenberg, N. 2006. Cap-dependent translational inhibition establishes two opposing morphogen gradients in Drosophila embryos. *Curr Biol* **16**(20): 2035-2041.

Church, D.L., Guan, K.L., and Lambie, E.J. 1995. Three genes of the MAP kinase cascade, mek-2, mpk-1/sur-1 and let-60 ras, are required for meiotic cell cycle progression in Caenorhabditis elegans. *Development* **121**(8): 2525-2535.

Ciosk, R., DePalma, M., and Priess, J.R. 2004. ATX-2, the C. elegans ortholog of ataxin 2, functions in translational regulation in the germline. *Development* **131**(19): 4831-4841.

Ciosk, R., DePalma, M., and Priess, J.R. 2006. Translational regulators maintain totipotency in the Caenorhabditis elegans germline. *Science* **311**(5762): 851-853.

Clouse, K.N., Ferguson, S.B., and Schupbach, T. 2008. Squid, Cup, and PABP55B function together to regulate gurken translation in Drosophila. *Dev Biol* **313**(2): 713-724.

Coller, J. and Parker, R. 2005. General translational repression by activators of mRNA decapping. *Cell* **122**(6): 875-886.

Coller, J.M., Tucker, M., Sheth, U., Valencia-Sanchez, M.A., and Parker, R. 2001. The DEAD box helicase, Dhh1p, functions in mRNA decapping and interacts with both the decapping and deadenylase complexes. *RNA* **7**(12): 1717-1727.

Cougot, N., Babajko, S., and Seraphin, B. 2004. Cytoplasmic foci are sites of mRNA decay in human cells. *J Cell Biol* **165**(1): 31-40.

Crittenden, S.L., Bernstein, D.S., Bachorik, J.L., Thompson, B.E., Gallegos, M., Petcherski, A.G., Moulder, G., Barstead, R., Wickens, M., and Kimble, J. 2002. A conserved RNA-binding protein controls germline stem cells in Caenorhabditis elegans. *Nature* **417**(6889): 660-663.

Crittenden, S.L., Leonhard, K.A., Byrd, D.T., and Kimble, J. 2006. Cellular analyses of the mitotic region in the Caenorhabditis elegans adult germ line. *Mol Biol Cell* **17**(7): 3051-3061.

Crittenden, S.L., Troemel, E.R., Evans, T.C., and Kimble, J. 1994. GLP-1 is localized to the mitotic region of the C. elegans germ line. *Development* **120**(10): 2901-2911.

Cui, J., Sackton, K.L., Horner, V.L., Kumar, K.E., and Wolfner, M.F. 2008. Wispy, the Drosophila homolog of GLD-2, is required during oogenesis and egg activation. *Genetics* **178**(4): 2017-2029.

De Renzis, S., Elemento, O., Tavazoie, S., and Wieschaus, E.F. 2007. Unmasking activation of the zygotic genome using chromosomal deletions in the Drosophila embryo. *PLoS Biol* **5**(5): e117.

Decker, C.J., Teixeira, D., and Parker, R. 2007. Edc3p and a glutamine/asparagine-rich domain of Lsm4p function in processing body assembly in Saccharomyces cerevisiae. *J Cell Biol* **179**(3): 437-449.

Deng, Y., Singer, R.H., and Gu, W. 2008. Translation of ASH1 mRNA is repressed by Puf6p-Fun12p/eIF5B interaction and released by CK2 phosphorylation. *Genes Dev* **22**(8): 1037-1050.

DeRenzo, C., Reese, K.J., and Seydoux, G. 2003. Exclusion of germ plasm proteins from somatic lineages by cullin-dependent degradation. *Nature* **424**(6949): 685-689.

Ebersole, T.A., Chen, Q., Justice, M.J., and Artzt, K. 1996. The quaking gene product necessary in embryogenesis and myelination combines features of RNA binding and signal transduction proteins. *Nat Genet* **12**(3): 260-265.

Eckmann, C.R., Crittenden, S.L., Suh, N., and Kimble, J. 2004. GLD-3 and control of the mitosis/meiosis decision in the germline of Caenorhabditis elegans. *Genetics* **168**(1): 147-160.

Eckmann, C.R., Kraemer, B., Wickens, M., and Kimble, J. 2002. GLD-3, a bicaudal-C homolog that inhibits FBF to control germline sex determination in C. elegans. *Dev Cell* **3**(5): 697-710.

Edenfeld, G., Volohonsky, G., Krukkert, K., Naffin, E., Lammel, U., Grimm, A., Engelen, D., Reuveny, A., Volk, T., and Klambt, C. 2006. The splicing factor crooked neck associates with the RNA-binding protein HOW to control glial cell maturation in Drosophila. *Neuron* **52**(6): 969-980.

Ephrussi, A., Dickinson, L.K., and Lehmann, R. 1991. Oskar organizes the germ plasm and directs localization of the posterior determinant nanos. *Cell* **66**(1): 37-50.

Ephrussi, A. and Lehmann, R. 1992. Induction of germ cell formation by oskar. *Nature* **358**(6385): 387-392.

Eulalio, A., Behm-Ansmant, I., Schweizer, D., and Izaurralde, E. 2007. P-body formation is a consequence, not the cause, of RNA-mediated gene silencing. *Mol Cell Biol* **27**(11): 3970-3981.

Fan, X.C. and Steitz, J.A. 1998. Overexpression of HuR, a nuclear-cytoplasmic shuttling protein, increases the in vivo stability of ARE-containing mRNAs. *EMBO J* **17**(12): 3448-3460.

Ferraiuolo, M.A., Basak, S., Dostie, J., Murray, E.L., Schoenberg, D.R., and Sonenberg, N. 2005. A role for the eIF4E-binding protein 4E-T in P-body formation and mRNA decay. *J Cell Biol* **170**(6): 913-924.

Flaherty, S.M., Fortes, P., Izaurralde, E., Mattaj, I.W., and Gilmartin, G.M. 1997. Participation of the nuclear cap binding complex in pre-mRNA 3' processing. *Proc Natl Acad Sci U S A* **94**(22): 11893-11898.

Flemr, M., Ma, J., Schultz, R.M., and Svoboda, P. P-body loss is concomitant with formation of a messenger RNA storage domain in mouse oocytes. *Biol Reprod* **82**(5): 1008-1017.

Forbes, A. and Lehmann, R. 1998. Nanos and Pumilio have critical roles in the development and function of Drosophila germline stem cells. *Development* **125**(4): 679-690.

Francis, R., Barton, M.K., Kimble, J., and Schedl, T. 1995. gld-1, a tumor suppressor gene required for oocyte development in Caenorhabditis elegans. *Genetics* **139**(2): 579-606.

Franks, T.M. and Lykke-Andersen, J. 2007. TTP and BRF proteins nucleate processing body formation to silence mRNAs with AU-rich elements. *Genes Dev* **21**(6): 719-735.

Fujiwara, Y., Komiya, T., Kawabata, H., Sato, M., Fujimoto, H., Furusawa, M., and Noce, T. 1994. Isolation of a DEAD-family protein gene that encodes a murine homolog of Drosophila vasa and its specific expression in germ cell lineage. *Proc Natl Acad Sci U S A* **91**(25): 12258-12262.

Galarneau, A. and Richard, S. 2005. Target RNA motif and target mRNAs of the Quaking STAR protein. *Nat Struct Mol Biol* **12**(8): 691-698.

Gallo, C.M., Munro, E., Rasoloson, D., Merritt, C., and Seydoux, G. 2008. Processing bodies and germ granules are distinct RNA granules that interact in C. elegans embryos. *Dev Biol* **323**(1): 76-87.

Gebauer, F., Xu, W., Cooper, G.M., and Richter, J.D. 1994. Translational control by cytoplasmic polyadenylation of c-mos mRNA is necessary for oocyte maturation in the mouse. *EMBO J* **13**(23): 5712-5720.

Giraldez, A.J., Mishima, Y., Rihel, J., Grocock, R.J., Van Dongen, S., Inoue, K., Enright, A.J., and Schier, A.F. 2006. Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs. *Science* **312**(5770): 75-79.

Goldstrohm, A.C., Hook, B.A., Seay, D.J., and Wickens, M. 2006. PUF proteins bind Pop2p to regulate messenger RNAs. *Nat Struct Mol Biol* **13**(6): 533-539.

Goodwin, E.B., Okkema, P.G., Evans, T.C., and Kimble, J. 1993. Translational regulation of tra-2 by its 3' untranslated region controls sexual identity in C. elegans. *Cell* **75**(2): 329-339.

Gouw, J.W., Pinkse, M.W., Vos, H.R., Moshkin, Y., Verrijzer, C.P., Heck, A.J., and Krijgsveld, J. 2009. In vivo stable isotope labeling of fruit flies reveals post-transcriptional regulation in the maternal-to-zygotic transition. *Mol Cell Proteomics* **8**(7): 1566-1578.

Gray, N.K. and Hentze, M.W. 1994. Iron regulatory protein prevents binding of the 43S translation pre-initiation complex to ferritin and eALAS mRNAs. *EMBO J* **13**(16): 3882-3891.

Gruidl, M.E., Smith, P.A., Kuznicki, K.A., McCrone, J.S., Kirchner, J., Roussell, D.L., Strome, S., and Bennett, K.L. 1996. Multiple potential germ-line helicases are components of the germ-line-specific P granules of Caenorhabditis elegans. *Proc Natl Acad Sci U S A* **93**(24): 13837-13842.

Guven-Ozkan, T., Nishi, Y., Robertson, S.M., and Lin, R. 2008. Global transcriptional repression in C. elegans germline precursors by regulated sequestration of TAF-4. *Cell* **135**(1): 149-160.

Guven-Ozkan, T., Robertson, S.M., Nishi, Y., and Lin, R. 2010. zif-1 translational repression defines a second, mutually exclusive OMA function in germline transcriptional repression. *Development* **137**(20): 3373-3382.

Gygi, S.P., Rochon, Y., Franza, B.R., and Aebersold, R. 1999. Correlation between protein and mRNA abundance in yeast. *Mol Cell Biol* **19**(3): 1720-1730.

Hachet, O. and Ephrussi, A. 2004. Splicing of oskar RNA in the nucleus is coupled to its cytoplasmic localization. *Nature* **428**(6986): 959-963.

Hafner, M., Landthaler, M., Burger, L., Khorshid, M., Hausser, J., Berninger, P., Rothballer, A., Ascano, M., Jr., Jungkamp, A.C., Munschauer, M. et al. 2010. Transcriptome-wide identification of RNA-binding protein and microRNA target sites by PAR-CLIP. *Cell* **141**(1): 129-141.

Hansen, D., Wilson-Berry, L., Dang, T., and Schedl, T. 2004. Control of the proliferation versus meiotic development decision in the C. elegans germline through regulation of GLD-1 protein accumulation. *Development* **131**(1): 93-104.

Hardy, R.J., Loushin, C.L., Friedrich, V.L., Jr., Chen, Q., Ebersole, T.A., Lazzarini, R.A., and Artzt, K. 1996. Neural cell type-specific expression of QKI proteins is altered in quakingviable mutant mice. *J Neurosci* **16**(24): 7941-7949.

Hay, B., Jan, L.Y., and Jan, Y.N. 1988. A protein component of Drosophila polar granules is encoded by vasa and has extensive sequence similarity to ATP-dependent helicases. *Cell* **55**(4): 577-587.

Henderson, S.T., Gao, D., Lambie, E.J., and Kimble, J. 1994. lag-2 may encode a signaling ligand for the GLP-1 and LIN-12 receptors of C. elegans. *Development* **120**(10): 2913-2924.

Hocine, S., Singer, R.H., and Grunwald, D. 2010. RNA processing and export. *Cold Spring Harb Perspect Biol* **2**(12): a000752.

Hodgman, R., Tay, J., Mendez, R., and Richter, J.D. 2001. CPEB phosphorylation and cytoplasmic polyadenylation are catalyzed by the kinase IAK1/Eg2 in maturing mouse oocytes. *Development* **128**(14): 2815-2822.

Houseley, J., LaCava, J., and Tollervey, D. 2006. RNA-quality control by the exosome. *Nat Rev Mol Cell Biol* **7**(7): 529-539.

Huarte, J., Belin, D., Vassalli, A., Strickland, S., and Vassalli, J.D. 1987. Meiotic maturation of mouse oocytes triggers the translation and polyadenylation of dormant tissue-type plasminogen activator mRNA. *Genes Dev* **1**(10): 1201-1211.

Huttelmaier, S., Zenklusen, D., Lederer, M., Dictenberg, J., Lorenz, M., Meng, X., Bassell, G.J., Condeelis, J., and Singer, R.H. 2005. Spatial regulation of beta-actin translation by Src-dependent phosphorylation of ZBP1. *Nature* **438**(7067): 512-515.

Illmensee, K. and Mahowald, A.P. 1974. Transplantation of posterior polar plasm in Drosophila. Induction of germ cells at the anterior pole of the egg. *Proc Natl Acad Sci U S A* **71**(4): 1016-1020.

Izaurralde, E., Lewis, J., Gamberi, C., Jarmolowski, A., McGuigan, C., and Mattaj, I.W. 1995. A cap-binding protein complex mediating U snRNA export. *Nature* **376**(6542): 709-712.

Jackson, R.J., Hellen, C.U., and Pestova, T.V. 2010. The mechanism of eukaryotic translation initiation and principles of its regulation. *Nat Rev Mol Cell Biol* **11**(2): 113-127.

Jan, E., Motzny, C.K., Graves, L.E., and Goodwin, E.B. 1999. The STAR protein, GLD-1, is a translational regulator of sexual identity in Caenorhabditis elegans. *EMBO J* **18**(1): 258-269.

Jenny, A., Hachet, O., Zavorszky, P., Cyrklaff, A., Weston, M.D., Johnston, D.S., Erdelyi, M., and Ephrussi, A. 2006. A translation-independent role of oskar RNA in early Drosophila oogenesis. *Development* **133**(15): 2827-2833.

Jeske, M., Moritz, B., Anders, A., and Wahle, E. 2011. Smaug assembles an ATP-dependent stable complex repressing nanos mRNA translation at multiple levels. *EMBO J* **30**(1): 90-103.

Jones, A.R., Francis, R., and Schedl, T. 1996. GLD-1, a cytoplasmic protein essential for oocyte differentiation, shows stage- and sex-specific expression during Caenorhabditis elegans germline development. *Dev Biol* **180**(1): 165-183.

Jones, A.R. and Schedl, T. 1995. Mutations in gld-1, a female germ cell-specific tumor suppressor gene in Caenorhabditis elegans, affect a conserved

domain also found in Src-associated protein Sam68. *Genes Dev* **9**(12): 1491-1504.

Kadyrova, L.Y., Habara, Y., Lee, T.H., and Wharton, R.P. 2007. Translational control of maternal Cyclin B mRNA by Nanos in the Drosophila germline. *Development* **134**(8): 1519-1527.

Kalifa, Y., Huang, T., Rosen, L.N., Chatterjee, S., and Gavis, E.R. 2006. Glorund, a Drosophila hnRNP F/H homolog, is an ovarian repressor of nanos translation. *Dev Cell* **10**(3): 291-301.

Kawasaki, I., Shim, Y.H., Kirchner, J., Kaminker, J., Wood, W.B., and Strome, S. 1998. PGL-1, a predicted RNA-binding component of germ granules, is essential for fertility in C. elegans. *Cell* **94**(5): 635-645.

Kedersha, N., Stoecklin, G., Ayodele, M., Yacono, P., Lykke-Andersen, J., Fritzler, M.J., Scheuner, D., Kaufman, R.J., Golan, D.E., and Anderson, P. 2005. Stress granules and processing bodies are dynamically linked sites of mRNP remodeling. *J Cell Biol* **169**(6): 871-884.

Kedersha, N.L., Gupta, M., Li, W., Miller, I., and Anderson, P. 1999. RNA-binding proteins TIA-1 and TIAR link the phosphorylation of eIF-2 alpha to the assembly of mammalian stress granules. *J Cell Biol* **147**(7): 1431-1442.

Khurana, J.S. and Theurkauf, W. 2010. piRNAs, transposon silencing, and Drosophila germline development. *J Cell Biol* **191**(5): 905-913.

Kim, J.H. and Richter, J.D. 2006. Opposing polymerase-deadenylase activities regulate cytoplasmic polyadenylation. *Mol Cell* **24**(2): 173-183.

Kim, K.W., Nykamp, K., Suh, N., Bachorik, J.L., Wang, L., and Kimble, J. 2009. Antagonism between GLD-2 binding partners controls gamete sex. *Dev Cell* **16**(5): 723-733.

Kim, K.W., Wilson, T.L., and Kimble, J. 2010. GLD-2/RNP-8 cytoplasmic poly(A) polymerase is a broad-spectrum regulator of the oogenesis program. *Proc Natl Acad Sci U S A* **107**(40): 17445-17450.

Kim-Ha, J., Kerr, K., and Macdonald, P.M. 1995. Translational regulation of oskar mRNA by bruno, an ovarian RNA-binding protein, is essential. *Cell* **81**(3): 403-412.

Kimble, J. and Crittenden, S.L. 2007. Controls of germline stem cells, entry into meiosis, and the sperm/oocyte decision in Caenorhabditis elegans. *Annu Rev Cell Dev Biol* **23**: 405-433.

Kimble, J.E. and White, J.G. 1981. On the control of germ cell development in Caenorhabditis elegans. *Dev Biol* **81**(2): 208-219.

Kondo, T., Furuta, T., Mitsunaga, K., Ebersole, T.A., Shichiri, M., Wu, J., Artzt, K., Yamamura, K., and Abe, K. 1999. Genomic organization and expression analysis of the mouse qkI locus. *Mamm Genome* **10**(7): 662-669.

Krol, J., Loedige, I., and Filipowicz, W. 2010. The widespread regulation of microRNA biogenesis, function and decay. *Nat Rev Genet* **11**(9): 597-610.

Ladomery, M., Wade, E., and Sommerville, J. 1997. Xp54, the Xenopus homologue of human RNA helicase p54, is an integral component of stored mRNP particles in oocytes. *Nucleic Acids Res* **25**(5): 965-973.

Lai, W.S., Carballo, E., Strum, J.R., Kennington, E.A., Phillips, R.S., and Blackshear, P.J. 1999. Evidence that tristetraprolin binds to AU-rich elements and promotes the deadenylation and destabilization of tumor necrosis factor alpha mRNA. *Mol Cell Biol* **19**(6): 4311-4323.

Lamont, L.B., Crittenden, S.L., Bernstein, D., Wickens, M., and Kimble, J. 2004. FBF-1 and FBF-2 regulate the size of the mitotic region in the C. elegans germline. *Dev Cell* **7**(5): 697-707.

Larocque, D., Fragoso, G., Huang, J., Mushynski, W.E., Loignon, M., Richard, S., and Almazan, G. 2009. The QKI-6 and QKI-7 RNA binding proteins block proliferation and promote Schwann cell myelination. *PLoS One* **4**(6): e5867.

Larocque, D., Pilotte, J., Chen, T., Cloutier, F., Massie, B., Pedraza, L., Couture, R., Lasko, P., Almazan, G., and Richard, S. 2002. Nuclear retention of MBP mRNAs in the quaking viable mice. *Neuron* **36**(5): 815-829.

Lecuyer, E., Yoshida, H., Parthasarathy, N., Alm, C., Babak, T., Cerovina, T., Hughes, T.R., Tomancak, P., and Krause, H.M. 2007. Global analysis of mRNA localization reveals a prominent role in organizing cellular architecture and function. *Cell* **131**(1): 174-187.

Lee, M.H. and Schedl, T. 2001. Identification of in vivo mRNA targets of GLD-1, a maxi-KH motif containing protein required for C. elegans germ cell development. *Genes Dev* **15**(18): 2408-2420.

Lee, M.H. and Schedl, T. 2004. Translation repression by GLD-1 protects its mRNA targets from nonsense-mediated mRNA decay in C. elegans. *Genes Dev* **18**(9): 1047-1059.

Lehmann, R. and Nusslein-Volhard, C. 1986. Abdominal segmentation, pole cell formation, and embryonic polarity require the localized activity of oskar, a maternal gene in Drosophila. *Cell* **47**(1): 141-152.

Levy, S., Avni, D., Hariharan, N., Perry, R.P., and Meyuhas, O. 1991. Oligopyrimidine tract at the 5' end of mammalian ribosomal protein mRNAs is required for their translational control. *Proc Natl Acad Sci U S A* **88**(8): 3319-3323.

Li, Z., Takakura, N., Oike, Y., Imanaka, T., Araki, K., Suda, T., Kaname, T., Kondo, T., Abe, K., and Yamamura, K. 2003. Defective smooth muscle development in qkI-deficient mice. *Dev Growth Differ* **45**(5-6): 449-462.

Li, Z., Zhang, Y., Li, D., and Feng, Y. 2000. Destabilization and mislocalization of myelin basic protein mRNAs in quaking dysmyelination lacking the QKI RNA-binding proteins. *J Neurosci* **20**(13): 4944-4953.

Lin, H. and Spradling, A.C. 1997. A novel group of pumilio mutations affects the asymmetric division of germline stem cells in the Drosophila ovary. *Development* **124**(12): 2463-2476.

Lin, M.D., Jiao, X., Grima, D., Newbury, S.F., Kiledjian, M., and Chou, T.B. 2008. Drosophila processing bodies in oogenesis. *Dev Biol* **322**(2): 276-288.

Linder, P. 2006. Dead-box proteins: a family affair--active and passive players in RNP-remodeling. *Nucleic Acids Res* **34**(15): 4168-4180.

Liu, J., Valencia-Sanchez, M.A., Hannon, G.J., and Parker, R. 2005. MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies. *Nat Cell Biol* **7**(7): 719-723.

Long, R.M., Singer, R.H., Meng, X., Gonzalez, I., Nasmyth, K., and Jansen, R.P. 1997. Mating type switching in yeast controlled by asymmetric localization of ASH1 mRNA. *Science* **277**(5324): 383-387.

Lublin, A.L. and Evans, T.C. 2007. The RNA-binding proteins PUF-5, PUF-6, and PUF-7 reveal multiple systems for maternal mRNA regulation during C. elegans oogenesis. *Dev Biol* **303**(2): 635-649.

Lykke-Andersen, J. and Wagner, E. 2005. Recruitment and activation of mRNA decay enzymes by two ARE-mediated decay activation domains in the proteins TTP and BRF-1. *Genes Dev* **19**(3): 351-361.

Ma, J., Flemr, M., Stein, P., Berninger, P., Malik, R., Zavolan, M., Svoboda, P., and Schultz, R.M. 2010. MicroRNA activity is suppressed in mouse oocytes. *Curr Biol* **20**(3): 265-270.

Mair, G.R., Braks, J.A., Garver, L.S., Wiegant, J.C., Hall, N., Dirks, R.W., Khan, S.M., Dimopoulos, G., Janse, C.J., and Waters, A.P. 2006. Regulation of sexual development of Plasmodium by translational repression. *Science* **313**(5787): 667-669.

Mancebo, R., Zhou, X., Shillinglaw, W., Henzel, W., and Macdonald, P.M. 2001. BSF binds specifically to the bicoid mRNA 3' untranslated region and contributes to stabilization of bicoid mRNA. *Mol Cell Biol* **21**(10): 3462-3471.

Marin, V.A. and Evans, T.C. 2003. Translational repression of a C. elegans Notch mRNA by the STAR/KH domain protein GLD-1. *Development* **130**(12): 2623-2632.

Markesich, D.C., Gajewski, K.M., Nazimiec, M.E., and Beckingham, K. 2000. bicaudal encodes the Drosophila beta NAC homolog, a component of the ribosomal translational machinery*. *Development* **127**(3): 559-572.

Mello, C.C., Draper, B.W., Krause, M., Weintraub, H., and Priess, J.R. 1992. The pie-1 and mex-1 genes and maternal control of blastomere identity in early C. elegans embryos. *Cell* **70**(1): 163-176.

Mello, C.C., Schubert, C., Draper, B., Zhang, W., Lobel, R., and Priess, J.R. 1996. The PIE-1 protein and germline specification in C. elegans embryos. *Nature* **382**(6593): 710-712.

Merritt, C., Rasoloson, D., Ko, D., and Seydoux, G. 2008. 3' UTRs are the primary regulators of gene expression in the C. elegans germline. *Curr Biol* **18**(19): 1476-1482.

Merritt, C. and Seydoux, G. 2010. The Puf RNA-binding proteins FBF-1 and FBF-2 inhibit the expression of synaptonemal complex proteins in germline stem cells. *Development* **137**(11): 1787-1798.

Miller, M.A., Nguyen, V.Q., Lee, M.H., Kosinski, M., Schedl, T., Caprioli, R.M., and Greenstein, D. 2001. A sperm cytoskeletal protein that signals oocyte meiotic maturation and ovulation. *Science* **291**(5511): 2144-2147.

Minshall, N., Reiter, M.H., Weil, D., and Standart, N. 2007. CPEB interacts with an ovary-specific eIF4E and 4E-T in early Xenopus oocytes. *J Biol Chem* **282**(52): 37389-37401.

Minshall, N., Thom, G., and Standart, N. 2001. A conserved role of a DEAD box helicase in mRNA masking. *RNA* **7**(12): 1728-1742.

Mootz, D., Ho, D.M., and Hunter, C.P. 2004. The STAR/Maxi-KH domain protein GLD-1 mediates a developmental switch in the translational control of C. elegans PAL-1. *Development* **131**(14): 3263-3272.

Nabel-Rosen, H., Toledano-Katchalski, H., Volohonsky, G., and Volk, T. 2005. Cell divisions in the drosophila embryonic mesoderm are repressed via posttranscriptional regulation of string/cdc25 by HOW. *Curr Biol* **15**(4): 295-302.

Nabel-Rosen, H., Volohonsky, G., Reuveny, A., Zaidel-Bar, R., and Volk, T. 2002. Two isoforms of the Drosophila RNA binding protein, how, act in

opposing directions to regulate tendon cell differentiation. *Dev Cell* **2**(2): 183-193.

Nakamura, A., Amikura, R., Hanyu, K., and Kobayashi, S. 2001. Me31B silences translation of oocyte-localizing RNAs through the formation of cytoplasmic RNP complex during Drosophila oogenesis. *Development* **128**(17): 3233-3242.

Nakamura, A., Sato, K., and Hanyu-Nakamura, K. 2004. Drosophila cup is an eIF4E binding protein that associates with Bruno and regulates oskar mRNA translation in oogenesis. *Dev Cell* **6**(1): 69-78.

Nakanishi, T., Kubota, H., Ishibashi, N., Kumagai, S., Watanabe, H., Yamashita, M., Kashiwabara, S., Miyado, K., and Baba, T. 2006. Possible role of mouse poly(A) polymerase mGLD-2 during oocyte maturation. *Dev Biol* **289**(1): 115-126.

Nakanishi, T., Kumagai, S., Kimura, M., Watanabe, H., Sakurai, T., Kashiwabara, S., and Baba, T. 2007. Disruption of mouse poly(A) polymerase mGLD-2 does not alter polyadenylation status in oocytes and somatic cells. *Biochem Biophys Res Commun* **364**(1): 14-19.

Nelson, M.R., Leidal, A.M., and Smibert, C.A. 2004. Drosophila Cup is an eIF4E-binding protein that functions in Smaug-mediated translational repression. *EMBO J* **23**(1): 150-159.

Nishi, Y. and Lin, R. 2005. DYRK2 and GSK-3 phosphorylate and promote the timely degradation of OMA-1, a key regulator of the oocyte-to-embryo transition in C. elegans. *Dev Biol* **288**(1): 139-149.

Noble, S.L., Allen, B.L., Goh, L.K., Nordick, K., and Evans, T.C. 2008. Maternal mRNAs are regulated by diverse P body-related mRNP granules during early Caenorhabditis elegans development. *J Cell Biol* **182**(3): 559-572.

Ohno, G., Hagiwara, M., and Kuroyanagi, H. 2008. STAR family RNA-binding protein ASD-2 regulates developmental switching of mutually exclusive alternative splicing in vivo. *Genes Dev* **22**(3): 360-374.

Paris, J., Swenson, K., Piwnica-Worms, H., and Richter, J.D. 1991. Maturation-specific polyadenylation: in vitro activation by p34cdc2 and phosphorylation of a 58-kD CPE-binding protein. *Genes Dev* **5**(9): 1697-1708.

Parker, R. and Song, H. 2004. The enzymes and control of eukaryotic mRNA turnover. *Nat Struct Mol Biol* **11**(2): 121-127.

Paynton, B.V. 1998. RNA-binding proteins in mouse oocytes and embryos: expression of genes encoding Y box, DEAD box RNA helicase, and polyA binding proteins. *Dev Genet* **23**(4): 285-298.

Peled-Zehavi, H., Berglund, J.A., Rosbash, M., and Frankel, A.D. 2001. Recognition of RNA branch point sequences by the KH domain of splicing factor 1 (mammalian branch point binding protein) in a splicing factor complex. *Mol Cell Biol* **21**(15): 5232-5241.

Pepling, M.E., Wilhelm, J.E., O'Hara, A.L., Gephardt, G.W., and Spradling, A.C. 2007. Mouse oocytes within germ cell cysts and primordial follicles contain a Balbiani body. *Proc Natl Acad Sci U S A* **104**(1): 187-192.

Pillai, R.S., Bhattacharyya, S.N., Artus, C.G., Zoller, T., Cougot, N., Basyuk, E., Bertrand, E., and Filipowicz, W. 2005. Inhibition of translational initiation by Let-7 MicroRNA in human cells. *Science* **309**(5740): 1573-1576.

Pisarev, A.V., Hellen, C.U., and Pestova, T.V. 2007. Recycling of eukaryotic posttermination ribosomal complexes. *Cell* **131**(2): 286-299.

Qin, X., Ahn, S., Speed, T.P., and Rubin, G.M. 2007. Global analyses of mRNA translational control during early Drosophila embryogenesis. *Genome Biol* **8**(4): R63.

Racki, W.J. and Richter, J.D. 2006. CPEB controls oocyte growth and follicle development in the mouse. *Development* **133**(22): 4527-4537.

Reese, K.J., Dunn, M.A., Waddle, J.A., and Seydoux, G. 2000. Asymmetric segregation of PIE-1 in C. elegans is mediated by two complementary mechanisms that act through separate PIE-1 protein domains. *Mol Cell* **6**(2): 445-455.

Ross, A.F., Oleynikov, Y., Kislauskis, E.H., Taneja, K.L., and Singer, R.H. 1997. Characterization of a beta-actin mRNA zipcode-binding protein. *Mol Cell Biol* **17**(4): 2158-2165.

Rouget, C., Papin, C., Boureux, A., Meunier, A.C., Franco, B., Robine, N., Lai, E.C., Pelisson, A., and Simonelig, M. 2010. Maternal mRNA deadenylation and decay by the piRNA pathway in the early Drosophila embryo. *Nature* **467**(7319): 1128-1132.

Ruvinsky, I. and Meyuhas, O. 2006. Ribosomal protein S6 phosphorylation: from protein synthesis to cell size. *Trends Biochem Sci* **31**(6): 342-348.

Rybarska, A., Harterink, M., Jedamzik, B., Kupinski, A.P., Schmid, M., and Eckmann, C.R. 2009. GLS-1, a novel P granule component, modulates a network of conserved RNA regulators to influence germ cell fate decisions. *PLoS Genet* **5**(5): e1000494.

Ryder, S.P., Frater, L.A., Abramovitz, D.L., Goodwin, E.B., and Williamson, J.R. 2004. RNA target specificity of the STAR/GSG domain post-transcriptional regulatory protein GLD-1. *Nat Struct Mol Biol* **11**(1): 20-28.

Saccomanno, L., Loushin, C., Jan, E., Punkay, E., Artzt, K., and Goodwin, E.B. 1999. The STAR protein QKI-6 is a translational repressor. *Proc Natl Acad Sci U S A* **96**(22): 12605-12610.

Salles, F.J., Lieberfarb, M.E., Wreden, C., Gergen, J.P., and Strickland, S. 1994. Coordinate initiation of Drosophila development by regulated polyadenylation of maternal messenger RNAs. *Science* **266**(5193): 1996-1999.

Schmid, M., Kuchler, B., and Eckmann, C.R. 2009. Two conserved regulatory cytoplasmic poly(A) polymerases, GLD-4 and GLD-2, regulate meiotic progression in C. elegans. *Genes Dev* **23**(7): 824-836.

Schumacher, B., Hanazawa, M., Lee, M.H., Nayak, S., Volkmann, K., Hofmann, E.R., Hengartner, M., Schedl, T., and Gartner, A. 2005. Translational repression of C. elegans p53 by GLD-1 regulates DNA damage-induced apoptosis. *Cell* **120**(3): 357-368.

Sen, G.L. and Blau, H.M. 2005. Argonaute 2/RISC resides in sites of mammalian mRNA decay known as cytoplasmic bodies. *Nat Cell Biol* **7**(6): 633-636.

Seydoux, G. and Dunn, M.A. 1997. Transcriptionally repressed germ cells lack a subpopulation of phosphorylated RNA polymerase II in early embryos of Caenorhabditis elegans and Drosophila melanogaster. *Development* **124**(11): 2191-2201.

Seydoux, G. and Fire, A. 1994. Soma-germline asymmetry in the distributions of embryonic RNAs in Caenorhabditis elegans. *Development* **120**(10): 2823-2834.

Seydoux, G., Mello, C.C., Pettitt, J., Wood, W.B., Priess, J.R., and Fire, A. 1996. Repression of gene expression in the embryonic germ lineage of C. elegans. *Nature* **382**(6593): 713-716.

Shaw, G. and Kamen, R. 1986. A conserved AU sequence from the 3' untranslated region of GM-CSF mRNA mediates selective mRNA degradation. *Cell* **46**(5): 659-667.

Sheets, M.D., Wu, M., and Wickens, M. 1995. Polyadenylation of c-mos mRNA as a control point in Xenopus meiotic maturation. *Nature* **374**(6522): 511-516.

Sheth, U. and Parker, R. 2003. Decapping and decay of messenger RNA occur in cytoplasmic processing bodies. *Science* **300**(5620): 805-808.

-. 2006. Targeting of aberrant mRNAs to cytoplasmic processing bodies. *Cell* **125**(6): 1095-1109.

Sidman, R.L., Dickie, M.M., and Appel, S.H. 1964. Mutant Mice (Quaking and Jimpy) with Deficient Myelination in the Central Nervous System. *Science* **144**: 309-311.

Silvera, D., Formenti, S.C., and Schneider, R.J. 2010. Translational control in cancer. *Nat Rev Cancer* **10**(4): 254-266.

Smibert, C.A., Wilson, J.E., Kerr, K., and Macdonald, P.M. 1996. smaug protein represses translation of unlocalized nanos mRNA in the Drosophila embryo. *Genes Dev* **10**(20): 2600-2609.

Spike, C.A., Bader, J., Reinke, V., and Strome, S. 2008. DEPS-1 promotes P-granule assembly and RNA interference in C. elegans germ cells. *Development* **135**(5): 983-993.

Spirin, A.S. 1966. "Masked" forms of mRNA. *Curr Top Dev Biol* **1**: 1-38.

Stalder, L. and Muhlemann, O. 2009. Processing bodies are not required for mammalian nonsense-mediated mRNA decay. *RNA* **15**(7): 1265-1273.

Stebbins-Boaz, B., Cao, Q., de Moor, C.H., Mendez, R., and Richter, J.D. 1999. Maskin is a CPEB-associated factor that transiently interacts with elF-4E. *Mol Cell* **4**(6): 1017-1027.

Stebbins-Boaz, B., Hake, L.E., and Richter, J.D. 1996. CPEB controls the cytoplasmic polyadenylation of cyclin, Cdk2 and c-mos mRNAs and is necessary for oocyte maturation in Xenopus. *EMBO J* **15**(10): 2582-2592.

Suh, N., Baehner, L., Moltzahn, F., Melton, C., Shenoy, A., Chen, J., and Blelloch, R. 2010. MicroRNA function is globally suppressed in mouse oocytes and early embryos. *Curr Biol* **20**(3): 271-277.

Suh, N., Crittenden, S.L., Goldstrohm, A., Hook, B., Thompson, B., Wickens, M., and Kimble, J. 2009. FBF and its dual control of gld-1 expression in the Caenorhabditis elegans germline. *Genetics* **181**(4): 1249-1260.

Suh, N., Jedamzik, B., Eckmann, C.R., Wickens, M., and Kimble, J. 2006. The GLD-2 poly(A) polymerase activates gld-1 mRNA in the Caenorhabditis elegans germ line. *Proc Natl Acad Sci U S A* **103**(41): 15108-15112.

Sutton, M.A. and Schuman, E.M. 2006. Dendritic protein synthesis, synaptic plasticity, and memory. *Cell* **127**(1): 49-58.

Tadros, W., Goldman, A.L., Babak, T., Menzies, F., Vardy, L., Orr-Weaver, T., Hughes, T.R., Westwood, J.T., Smibert, C.A., and Lipshitz, H.D. 2007.

SMAUG is a major regulator of maternal mRNA destabilization in Drosophila and its translation is activated by the PAN GU kinase. *Dev Cell* **12**(1): 143-155.

Tadros, W., Houston, S.A., Bashirullah, A., Cooperstock, R.L., Semotok, J.L., Reed, B.H., and Lipshitz, H.D. 2003. Regulation of maternal transcript destabilization during egg activation in Drosophila. *Genetics* **164**(3): 989-1001.

Takizawa, P.A., Sil, A., Swedlow, J.R., Herskowitz, I., and Vale, R.D. 1997. Actin-dependent localization of an RNA encoding a cell-fate determinant in yeast. *Nature* **389**(6646): 90-93.

Tarun, S.Z., Jr. and Sachs, A.B. 1996. Association of the yeast poly(A) tail binding protein with translation initiation factor eIF-4G. *EMBO J* **15**(24): 7168-7177.

Thompson, B.E., Bernstein, D.S., Bachorik, J.L., Petcherski, A.G., Wickens, M., and Kimble, J. 2005. Dose-dependent control of proliferation and sperm specification by FOG-1/CPEB. *Development* **132**(15): 3471-3481.

Thompson, S.R., Goodwin, E.B., and Wickens, M. 2000. Rapid deadenylation and Poly(A)-dependent translational repression mediated by the Caenorhabditis elegans tra-2 3' untranslated region in Xenopus embryos. *Mol Cell Biol* **20**(6): 2129-2137.

Thomsen, S., Anders, S., Janga, S.C., Huber, W., and Alonso, C.R. 2010. Genome-wide analysis of mRNA decay patterns during early Drosophila development. *Genome Biol* **11**(9): R93.

Toledano-Katchalski, H., Nir, R., Volohonsky, G., and Volk, T. 2007. Post-transcriptional repression of the Drosophila midkine and pleiotrophin homolog miple by HOW is essential for correct mesoderm spreading. *Development* **134**(19): 3473-3481.

Vassalli, J.D., Huarte, J., Belin, D., Gubler, P., Vassalli, A., O'Connell, M.L., Parton, L.A., Rickles, R.J., and Strickland, S. 1989. Regulated polyadenylation controls mRNA translation during meiotic maturation of mouse oocytes. *Genes Dev* **3**(12B): 2163-2171.

Voeltz, G.K. and Steitz, J.A. 1998. AUUUA sequences direct mRNA deadenylation uncoupled from decay during Xenopus early development. *Mol Cell Biol* **18**(12): 7537-7545.

Wang, C. and Lehmann, R. 1991. Nanos is the localized posterior determinant in Drosophila. *Cell* **66**(4): 637-647.

Wang, L., Eckmann, C.R., Kadyk, L.C., Wickens, M., and Kimble, J. 2002. A regulatory cytoplasmic poly(A) polymerase in Caenorhabditis elegans. *Nature* **419**(6904): 312-316.

Wang, X., Li, W., Williams, M., Terada, N., Alessi, D.R., and Proud, C.G. 2001. Regulation of elongation factor 2 kinase by p90(RSK1) and p70 S6 kinase. *EMBO J* **20**(16): 4370-4379.

Wang, X., Zhao, Y., Wong, K., Ehlers, P., Kohara, Y., Jones, S.J., Marra, M.A., Holt, R.A., Moerman, D.G., and Hansen, D. 2009. Identification of genes expressed in the hermaphrodite germ line of C. elegans using SAGE. *BMC Genomics* **10**: 213.

Wilhelm, J.E., Buszczak, M., and Sayles, S. 2005. Efficient protein trafficking requires trailer hitch, a component of a ribonucleoprotein complex localized to the ER in Drosophila. *Dev Cell* **9**(5): 675-685.

Wreden, C., Verrotti, A.C., Schisa, J.A., Lieberfarb, M.E., and Strickland, S. 1997. Nanos and pumilio establish embryonic polarity in Drosophila by promoting posterior deadenylation of hunchback mRNA. *Development* **124**(15): 3015-3023.

Wright, J.E., Gaidatzis, D., Senften, M., Farley, B.M., Westhof, E., Ryder, S.P., and Ciosk, R. 2010. A quantitative RNA code for mRNA target selection by the germline fate determinant GLD-1. *EMBO J*.

Wu, J., Zhou, L., Tonissen, K., Tee, R., and Artzt, K. 1999. The quaking I-5 protein (QKI-5) has a novel nuclear localization signal and shuttles between the nucleus and the cytoplasm. *J Biol Chem* **274**(41): 29202-29210.

Wu, J.I., Reed, R.B., Grabowski, P.J., and Artzt, K. 2002. Function of quaking in myelination: regulation of alternative splicing. *Proc Natl Acad Sci U S A* **99**(7): 4233-4238.

Yano, T., Lopez de Quinto, S., Matsui, Y., Shevchenko, A., and Ephrussi, A. 2004. Hrp48, a Drosophila hnRNPA/B homolog, binds and regulates translation of oskar mRNA. *Dev Cell* **6**(5): 637-648.

Zaessinger, S., Busseau, I., and Simonelig, M. 2006. Oskar allows nanos mRNA translation in Drosophila embryos by preventing its deadenylation by Smaug/CCR4. *Development* **133**(22): 4573-4583.

Zamore, P.D., Williamson, J.R., and Lehmann, R. 1997. The Pumilio protein binds RNA through a conserved domain that defines a new class of RNA-binding proteins. *RNA* **3**(12): 1421-1433.

Zhang, B., Gallegos, M., Puoti, A., Durkin, E., Fields, S., Kimble, J., and Wickens, M.P. 1997. A conserved RNA-binding protein that regulates sexual fates in the C. elegans hermaphrodite germ line. *Nature* **390**(6659): 477-484.

Zhang, F., Barboric, M., Blackwell, T.K., and Peterlin, B.M. 2003. A model of repression: CTD analogs and PIE-1 inhibit transcriptional elongation by P-TEFb. *Genes Dev* **17**(6): 748-758.

Zhang, S., Williams, C.J., Wormington, M., Stevens, A., and Peltz, S.W. 1999. Monitoring mRNA decapping activity. *Methods* **17**(1): 46-51.

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